An Investigation of the Relative Efficiencies of Natural and Artificial Propagation of Sockeye Salmon (*Oncorhynchus nerka*) at Cultus Lake, British Columbia

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ABSTRACT

From three tests each of natural propagation and of artificial propagation involving liberation of free-swimming fry and two tests of artificial propagation involving planting of eyed eggs, it was found that no statistically significant difference occurred between the three methods. Computing the percentage efficiency as the number of seaward migrants produced from the total eggs handled, natural propagation varied from 1.05% to 3.23%—an average of 1.80%; fry liberation from 2.42% to 4.54%—an average of 3.24%; and egg planting from 1.45% to 4.71%—an average of 3.08%. Losses occurring during the five years' stripping and hatchery operations were recorded and, based on total eggs, the hatchery product available for distribution represented from 61.0% to 86.0% for egg planting and from 63.4% to 78.5% for fry liberation. There occurred a lake mortality of approximately 96% of the number of fry liberated. The conclusion was reached that in an area such as Cultus lake, where a natural run of sockeye occurred with a reasonable expectancy of successful spawning, artificial propagation, as commonly practised, provided no advantage over natural spawning, as a means of maintaining the run.

From 1925 to 1936 inclusive an investigation of the relative efficiencies of natural and of artificial propagation of sockeye salmon, (*Oncorhynchus nerka*), was carried out at Cultus lake, British Columbia. Two methods of artificial propagation were involved, namely, eyed egg planting and free-swimming fry distribution. The primary purpose was to determine whether either of these artificial measures actually provided any beneficial results as compared with natural spawning, and, if so, to what extent.

PLAN OF INVESTIGATION

In the initial paper of this series (Foerster 1929a) there is given a detailed explanation of the programme of the investigation. As far as possible this scheme has been followed and several reports, dealing with individual phases or complete cycles of the life history have been issued from time to time (Foerster 1929a, 1929b, 1929c, 1934 and 1936a).

The investigation was planned to extend over a period of twelve years, thus permitting four tests of each propagational method. The occurrence, however, of an exceedingly large spawning run of sockeye in each fourth year, entering the investigation in 1927 and 1931, so exceeded the capacity of the hatchery facilities

to handle it that natural propagation had to be resorted to. In 1927 a test of natural propagation was made, but in 1931 a portion of the run was handled artificially and the remainder, approximately one-half of the run, was allowed to spawn naturally. The results of the 1931 operations are not included in the investigation proper.

As the data from the several tests accumulated it became evident that very little difference was to be found between the three methods being tested. There seemed little object, therefore, in continuing the studies in order to obtain the fourth series of results. The investigation had by that time clearly indicated certain lines of research deserving more immediate attention. It was decided, therefore, to conclude the main investigation with the second test of eyed-egg planting in 1933.

Early presentation of the primary results obtained seemed desirable and accordingly the data were reported in brief form (Foerster 1936c). The present paper is designed to present the accumulated data in greater detail and refer to certain features which were of necessity omitted from the earlier account.

METHODS

The results of the investigation are based primarily upon a computation of the "in-go" to the lake each year, i.e., the extent of spawning, and the "out-go", i.e., the extent of the seaward migration which subsequently occurs. Two counting fences, described and illustrated in Foerster (1936c), were erected below the lake, one for the counting of up-stream migrating spawning adults and for use in artificial stripping, etc., the other for the counting of downstream migrating young. In order that all tests be readily comparable it was at the outset decided that the "in-go" should be represented by the total number of eggs contained in all of the adult female sockeye arriving at the counting and stripping weir, whereas the "out-go" should be indicated by the number of fry, yearlings or two-year-old migrants counted migrating downstream from the lake.

The success or efficiency of any propagation test was based, therefore, upon the number of seaward-migrating young resulting from each particular method of propagation involved. These migrants consisted largely of one-year-old individuals, but on occasion migrations of fry and of two-year-olds occurred. In computing the product from any spawning, therefore, the occurrence of each class of migrant had to be considered, and, since each class migrated in different years, this was done by analyzing the constituents of each season's migration and crediting the various age-classes to their appropriate spawning or brood year.

The enumeration of fry migrants presented no difficulty and could be readily accomplished. The over-lapping in size, in certain years, between the two older age-groups in migration made necessary, however, a sampling of the migration and the separation of the sample individuals according to age, as determined by scale reading. In 1927 the samples were obtained by dipping out from time to time (Foerster 1929c) random collections from the counting pens, but in subsequent years the practice was to preserve one individual for each fifty, one hundred, two hundred or five hundred migrants counted during the migration season.

For each year's migration the numbers of individuals of each of the two age-classes in the sample were ascertained, and from the proportions thus prevailing the total migrants of each age-group in the entire migration were computed. In table I are tabulated the data for each season's migration, together with the extent of the sampling and the separation of migrants into the two age-groups. For each migration the fiducial limits of confidence, on the basis of a confidence coefficient of 0.99, are given for both age-classes.

TABLE I. Data pertaining to the number of seaward migrants contained in each migration, the extent of the sampling, the treatment of the sample to separate the two age-classes and the calculated fiducial limits of each age group.

Year of migration	1 9 2 7	1 9 2 8	1 9 2 9	1 9 3 0	1 9 3 1	1 9 3 2	1 9 3 3	1 9 3 4	1 9 3 5	1 9 3 6
Total migration	249,961	337,895	2,484,556	178,253	355,149	788,637	1,545,190	184,854	256,708	502,983
Number in sample	261	2,186	4,792	1,192	3,412	3,709	1,552	1,113	1,625	5,340
Yearlings	187	2,172	4,761	408	3,362	3,708	1,536	609	1,514	5,331
Two-year-olds	74	14	31	784	50	1	16	504	111	9
Size of sample used to calculate the fiducial limits	200.0		3,697	1,052				919	1,275	
Yearlings	146.6		3,686	386				604.7	1,204.2	
Two-year-olds	53.4		11	666				314.3	70.8	
Most probable number of yearlings	183,400	336,200	2,456,200	38,600	349,900	788,400	1,529,300	121,500	242,500	501,600
Fiducial limits of yearlings	161,100 to 206,000	333,800 to 338,900	2,449,300 to 2,459,300	34,600 to 42,600	347,700 to 351,600	787,100 to 788,600	1,515,900 to 1,537,700	114,100 to 129,000	238,100 to 246,600	499,700 to 501,400
Most probable number of two-year-olds	66,500	1,700	8,300	66,600	5,200	200	15,900	63,400	14,200	2,200
Fiducial limits of two-year-olds	44,100 to 89,100	1,000 to 4,100	7,900 to 15,200	62,600 to 70,700	3,500 to 7,400	0 to 1,600	7,500 to 29,300	55,900 to 63,400	10,100 to 18,500	1,800 to 3,300

In some years, e.g., 1927, 1929, 1930, 1934, 1935, when the ratio of sample to migration varied during the season, exact calculation of the fiducial limits was not possible. Limits have, however, been calculated on the basis of the smallest ratio used, giving a safe estimate of the accuracy. In other years, e.g., 1928, 1931, 1932, 1933 and 1936, the sampling was uniform and permitted a direct calculation of fiducial limits. In all cases the limits of confidence have been derived by the methods outlined by Clopper and Pearson (1934) and Ricker (1937).

In the great majority of cases, utilization of the fiducial limits changes only very slightly the percentages obtained for the most probable numbers. No attempt has been made, therefore, to indicate the range of percentage efficiency as set by these limits. Indeed, the range of variation exhibited by the sampling error of the mean egg counts each year contributes more to the possible statistical error in percentage efficiency than do the fiducial limits of the seaward-migrant sampling.

RESULTS

The data accumulated for each test in the three methods of propagation investigated are given in tables II and III. In each case the pertinent spawning records are set down, followed by the counts of each class of migrants subsequently enumerated. Discussion of particular features of the tests will be found in the appropriate sections.

NATURAL PROPAGATION

In the three tests of natural spawning, table II, *the percentage efficiency is found to vary from one to three per cent, with an average of 1.80 per cent.*

TABLE II. Data pertaining to each of the three tests of natural propagation at Cultus lake, British Columbia

Year	1925	1927	1930
Number of males counted	1,540	26,049	4,853
Number of females counted	3,883	56,376	5,542
Average egg content per female	4,500	4,500	4,500
Females released to spawn	3,883	55,569	5,542
Eggs available for deposition	17,470,000	250,000,000	24,900,000
Migrants: Fry	12,600	118,600	0
Yearlings	183,400	2,456,200	788,400
Two-year-olds	1,700	66,600	15,900
Total	197,700	2,641,400	804,300
Migrants as per cent of total eggs:	1.13	1.04	3.23
Migrants as per cent of eggs available for deposition:	1.13	1.05	3.23

It will be observed that in each of the three years the ratio of sexes, males to females, varied appreciably and that in 1930, the year when the sexes were nearest to equality, the percentage efficiency was highest. To what extent the paucity of males in the other two years has affected the successful fertilization of the quantities of eggs presumed to have been deposited and suitably fertilized remains a matter of conjecture, but in correlating the number of males to the percentage efficiency obtained for each of the years a coefficient of 0.99 was obtained with a probability (Fisher 1932) of between 0.1 and 0.05. This, though not significant, may be indicative of a definite relationship between the fertilizing capacity of the male population, when in the minority, and the number of seaward migrants produced.

If calculations of percentage efficiency of natural propagation are based upon the eggs contained in that number of females equivalent to the number of males, the percentages are raised to 2.86, 2.25 and 3.47 respectively, the average being 2.86 per cent. The statistics of the commercial catches and the reports from other sockeye spawning areas in British Columbia (Clemens 1935) indicate that the sexes are usually equal in number or the males slightly in excess. The proportions of sexes at Cultus lake may possibly result in a low efficiency of production and the percentages obtained may, therefore, represent minimal ones for natural propagation.

DEPOSITION OF EGGS

With reference to the mortality occurring at intermediate stages between the deposition of eggs and the migration seaward, very little information was revealed. Interference with spawning fish and possible damage to spawning nests, which

might be occasioned by attempts to obtain data on these points, had to be assiduously avoided. Nevertheless, certain records were collected with respect to the completeness with which eggs are naturally expressed. For the two years in which spawned-out female sockeye were examined on the spawning beds the counts of eggs remaining in the body cavity of the spawners were:

No. eggs retained No. fish	0	1 to 20	21 to 50	51 to 100	101 to 300	301 to 600	601 to 1300
1925	22	21	3	3	2	2	1
1935	126	206	22	17	32	12	2

Mean counts for the 54 samples of 1925 and the 417 of 1936 were 47.4 ± 14.1 and 36.5 ± 3.52 respectively. In both instances over 75 per cent of the specimens examined contained 20 or fewer eggs per fish. It would seem, therefore, that practically complete deposition of eggs by the female occurs.

Not included in the above records are the recoveries of three females in 1925 and 99 in 1935 found dead and unspawned. Causes of death are unknown. Whether or not they resulted from the scarcity of males and the consequent inability to mate remains in doubt. They may tend to offer some explanation for the correlation above between males and percentage efficiency of natural propagation.

FERTILIZATION OF EGGS

For information concerning the extent of fertilization under natural spawning conditions, again interference with the spawning beds was not deemed advisable. Recourse was had to experimentation.

In Foerster (1936b) the results are reported of experiments in which partially-stripped female sockeye were placed in prepared pens and allowed to deposit the remainder of their eggs. For Cultus lake tests deposition and fertilization amounted to 77.2 per cent, whereas in a number of experiments conducted by the Department of Fisheries, approximately 65 per cent successful deposition and fertilization was obtained.

During the 1934-35 season further experiments were carried out at Cultus lake in which mature and unspawned females were placed in prepared cages with gravel bottoms, supplied with males and allowed to spawn naturally. Two pens were used and one pair of sockeye was placed in each.

When the females died they were removed and a count made of the eggs left in the body cavity. In one there were two eggs; in the other, nine. In the spring of 1935 the gravel of the pen bottoms was turned over and eggs and fry picked out, as follows: Pen no. 1—2,279 live eggs, 68 dead eggs and 63 fry; pen no. 2—3,370 live eggs, 32 dead eggs and 53 fry. Unfortunately high water prevented examination of the gravel before a considerable number of the eggs had hatched and the results obtained are, therefore, strictly minimal. Based on an average egg content per female of 4,282 eggs—the mean for the 1934 spawning—the results show a successful development of 55 per cent for the first pen and 80 per cent for the second.

From the several general tests, therefore, it would appear that under natural conditions the successful deposition, fertilization and development of eggs would approximate 70 per cent.

For Kamchatka sockeye Krokhin and Krogius (1937) report an average mortality of 36.7 per cent during natural propagation at lake Kuril or a survival of approximately 63 per cent. Rutter (1904) records "probably over 80 per cent" fertilization for Quinnat salmon (*O. tschawytscha*) of California, while Hobbs (1937) reports, for the same species in New Zealand, a hatch, under favourable conditions, of over 97 per cent of ova lodged in redds sampled in 1933. Shapovalov (1937) indicates a high degree of fertilization and hatch under natural conditions for steelhead trout (*Salmo gairdneri*) in California.

Artificial Propagation

In table III the pertinent data for all of the tests of artificial propagation are presented, including stripping of the eggs and hatchery incubation, prior to the distribution of fry or planting of "eyed" eggs.

Table III. Data pertaining to each of the three tests of artificial propagation with liberation of free-swimming fry and each of the two tests of artificial propagation with planting of "eyed" eggs.

Year	1926	1929	1932	1928	1933
Number of males counted	3,122	1,645	741	3,878	1,565
Number of females counted	1,949	3,437	1,518	11,461	1,906
Average egg content per female	4,500	4,545±39	4,324±70	4,511±45	3,600±42
Total eggs available	8,770,500	15,620,000	6,564,000	51,700,000	7,243,000
Females recovered dead	541	472	445	3,689	456
Egg loss involved	1,174,500	2,144,000	1,924,000	17,543,000	1,733,000
Eggs available for stripping	7,596,000	13,476,000	4,640,000	34,157,000	5,510,000
Eggs unrecovered in stripping	1,109,000	1,397,000	136,300	1,500,000	554,500
Eggs collected	6,487,000	12,079,000	4,503,700	32,657,000 (5,985,300)***	4,955,000
Losses in hatchery	570,500	2,435,000	505,700	487,900	320,300
Total eggs or fry for distribution	5,916,500	9,644,000	3,998,000	5,497,400	4,635,200
Per cent of total eggs	67.5	61.7	60.9	58.0	64.0
Per cent of eggs available for stripping	77.9	71.5	86.2	87.8	84.1
Total eggs or fry distributed	5,916,500	9,093,000**	4,824,900*	2,649,600***	4,371,500**
Migrants : Yearlings	336,200	349,900	100,700	38,600	242,500
Two-year-olds	8,300	200	11,800	5,200	2,200
Total	344,500	350,100	112,500	43,800	244,700
Migrants as per cent of : total eggs available	3.90	2.38	1.71	0.98	3.38
eggs available for stripping	4.54	2.76	2.42	1.45	4.71
eggs or fry distributed	5.83	3.85	2.81	1.65	5.82

* Additional eggs or fry were available for distribution. Percentages are computed to account for same.
** Eggs or fry were taken for other experiments. Percentages computed make allowance for same.
*** The planting areas being limited, most of the eggs were removed to other areas. Percentages are derived by calculating back from eggs planted, making use of the percentage loss in each preceding stage.

LOSSES IN EGGS

Mortality of adult females. At Cultus lake stripping of eggs was conducted in the outlet stream below the lake. The ascending spawners had, therefore, to be obstructed before they reached the lake where they normally would have remained until fully mature and ready to occupy the spawning beds. No spawners whatever were allowed to enter the lake, all being held below the counting and spawning weir until in suitable condition for stripping. This necessarily involved a considerable period of retention,—roughly a month for the earliest arrivals but probably

around one to two weeks for the majority,—and during this time they were constantly fighting the weir in an effort to break through. Once stripping commenced and the pens into which the fish were collected were opened a varying amount of handling of the fish occurred as the "ripe" females were segregated and stripped.

Much of the loss in females was thus occasioned and the consequent wastage in eggs, computed as a percentage of total eggs contained in all females, varied from 13.4 per cent (in 1926) to 33.9 per cent (in 1928), the average for the five years being 22.8 per cent.

The rigorous conditions laid down of necessity by the investigation unquestionably accentuated the losses in wasted eggs, for under normal conditions of artificial propagation at Cultus lake, some, at least, of the early-arriving fish would have been allowed to proceed to the lake. A certain amount of retention has always been practised, however, and is necessitated by the site of the stripping operations. Under such circumstances, and having regard to the fact that in other areas where artificial propagation of sockeye salmon is practised the stripping is done above the lake and when the fish are approaching the spawning grounds in a more mature condition, it has been decided to include the losses in eggs in dead and unspawned females in the computation of the efficiency of artificial propagation only as indicating the lower limit. A further calculation of efficiency is made (in the succeeding section) on the basis of total eggs contained in adult females stripped, and the true value lies somewhere between the two.

Incomplete stripping. Special comparative tests of the efficiencies of the two methods of stripping practised at Cultus lake, namely "expression" and "expression and incision" were independently made (Foerster 1936b) and it was found that "through incomplete removal of all eggs, therefore, expression stripping produced an apparent loss of approximately 12 per cent more" than "expression and incision". These tests were made with equal numbers of fish in relatively the same stage of maturity.

In the present investigation, however, involving handling of much greater numbers of eggs, in varying degrees of "ripeness", the differences do not prove as clear-cut nor significant. The data are tabulated as follows:

Year	Eggs available for stripping	Method of stripping	Per cent loss in eggs as based on	
			Total eggs available	Eggs available for stripping
1926	7,596,000	Expression	12.6	14.6
1928	34,157,000	Exp. and inc.	2.9	4.4
1929	13,476,000	Exp. and inc.	9.0	10.4
1932	4,640,000	Exp. and inc.	2.1	2.9
1933	5,510,000	Exp. and inc.	7.7	10.1
Mean percentages			6.9 ± 1.3	8.5 ± 1.4

The method of stripping appears to have had little effect on the number of eggs unrecovered in the stripping operations and the mean percentages obtained are quite indicative of the wastage in eggs in this phase of the stripping procedure.

It is worthy of note that in those years when the loss in female sockeye during retention, 1928 and 1932, was high, as shown in table III, the losses due to incomplete stripping were the lowest. The period of retention thus had no deleterious effect upon the actual stripping of eggs.

Incubation losses. In the two tests of "eyed" egg planting in which the eggs were removed from the hatchery at the "eyed" stage, the losses from time of setting-down in the hatchery to the time of transfer amounted to 5.2 and 4.4 per cent, respectively, of total eggs available, and 7.8 and 5.8 per cent, respectively, of eggs available for stripping.

For fry production, on the other hand, there were slightly greater losses, e.g., 6.5, 15.6 and 7.7 per cent, respectively, of total eggs available, and 7.5, 18.1 and 10.9 per cent, respectively, of eggs available for stripping. The circumstances which produced the unusually high loss in 1929 could not be isolated, but in any season the variable effects of water-flow and water temperatures in the hatchery troughs, and of expertness in handling the incubating eggs on the part of the hatchery attendants, are of considerable significance.

EVALUATION OF HATCHERY OPERATION

In summing up the results of the stripping operations and hatchery incubation of the collected eggs there are tabulated below, for each test of fry liberation and of egg planting, the resultant hatchery product available for distribution. The hatchery product is expressed as the percentage of eyed eggs or of free-swimming fry successfully obtained from (1) the total number of eggs available and (2) the eggs available for stripping. As noted above, the true percentage lies somewhere between these two.

	Fry liberation			Egg planting	
Year	(1)	(2)	Year	(1)	(2)
1926	67.5	77.9	1928	58.0	87.8
1929	61.7	71.5	1933	64.0	84.1
1932	60.9	86.2			
Mean percentage	63.4	78.5	Mean percentage	61.0	86.0

In both series of tests notable variations occurred in the results obtained for the several years, caused primarily by wide variations in the amount of mortality experienced either in the stripping operations or during the period of incubation. For example, in the fry liberation tests the operations in 1932 were most successful, as far as percentage survival of eggs available for stripping is concerned. In 1926 the operations suffered a relatively heavy wastage in eggs due to incomplete stripping, while in 1929 there occurred a heavy loss in eggs during incubation. Similar variations may be observed in the tests of eyed egg planting.

Considering the statistical significance of the results there is no significant difference between the percentages of migration (table III) for fry liberation and egg planting whether computed on the basis of percentage of total eggs available, the averages being 2.66 and 2.27 per cent respectively, or of eggs available for

stripping, 3.24 and 3.08 per cent respectively. In general, therefore, for artificial propagation, when calculated on the basis of the total number of eggs available for stripping, the mortality during stripping and incubation of the eggs amounts to approximately twenty per cent, that is, there is roughly eighty per cent survival. If the results of the stripping and hatchery operations are based, however, upon the total number of eggs available in all sockeye arriving at the counting weir, the loss amounts to approximately 40 per cent, leaving a survival of but sixty per cent. The difference between the two methods of calculation is the approximate measure of the wastage in eggs occurring through female sockeye dying during the retention and stripping period. How much of this loss should be considered a justifiable claim against artificial propagation remains a matter of opinion.

COMPARISON OF NATURAL AND ARTIFICIAL PROPAGATION

In the case of artificial propagation two sets of percentages have been calculated, one covering the total eggs contained in all female sockeye counted, 2.51 ± 0.42, and the other involving only those eggs contained in the female sockeye available for stripping, 3.18 ± 0.47. In the latter case the mortality due to female sockeye dying unspawned or unstripped during retention has been ignored. For natural propagation, likewise, two sets of data are available, that obtained from the actual spawning, average—1.80 ± 0.48 per cent, and, secondly, that computed on an equality of sexes, average—2.86 ± 0.29 per cent.

For general comparative purposes the percentages of efficiency obtained from the different sets of calculations may be set down as:

	Natural propagation	Artificial propagation	Difference
(1)	1.80 ± 0.48	3.18 ± 0.47	1.38
(2)	1.80 ± 0.48	2.51 ± 0.42	0.71
(3)	2.86 ± 0.29	3.18 ± 0.47	0.32
(4)	2.86 ± 0.29	2.51 ± 0.42	-0.35

In the first comparison, with natural propagation at a probable minimum and artificial propagation considered from a very favourable standpoint, the difference amounts to 1.38 per cent or an increase of approximately eighty per cent over natural spawning. It cannot be considered, however, that any advantage would actually prevail as the difference between the two percentage survivals is not statistically significant. In the second comparison, where both natural and artificial propagation values are minimal ones, the difference is greatly reduced and very much less significant. In those instances, (3) and (4), where natural propagation percentages are considered maximum, the differences between natural and artificial methods are decidedly variable and highly non-significant. In fact, in the last comparison, artificial propagation is less efficient than natural.

Therefore, from a statistical analysis of the data obtained, it is readily apparent that artificial propagation exhibits no significantly increased efficiency, in point of seaward-migrating young sockeye, over natural spawning.

MORTALITY OF YOUNG IN LAKE

In table III are given the percentages of seaward migrants based on the actual numbers of fry placed in the lake. They are 5.83, 3.85 and 2.81, respectively, the

average being 4.16 ± 0.60. During that phase of the life history extending from the fry to the seaward migrant stage, for the most part embracing a period of a year, the mortality amounts to approximately 96 per cent. This occurs after the hatchery operations have terminated and thus pertains to the product of artificial propagation and natural spawning alike.

The revelation of the occurrence of a very great loss among sockeye fry in the lake indicates one line along which future investigation should proceed in the endeavour to determine means whereby the production of sockeye salmon may be increased.

SUMMARY

To determine the relative efficiencies of natural and artificial methods of propagation of sockeye salmon, an extensive investigation was commenced at Cultus lake, British Columbia, in 1925 and continued to 1936. By ascertaining the extent of the "in-go" to the lake under each system of propagation, represented by the number of eggs contained in the female sockeye handled, and the subsequent "out-go", in the form of seaward migrants, the efficiencies of the methods could be calculated.

For natural propagation the three tests gave percentage efficiencies of 1.13, 1.05 and 3.23 per cent of eggs presumed to have been deposited, or an average of 1.80 per cent. In two tests the male sockeye were appreciably deficient in numbers and consideration is given to the probable effect.

For artificial propagation, two methods were investigated, fry liberation and egg planting. The difference applied only to the means of disposition of the hatchery product and throughout the stripping operations and incubation of the eggs in the hatchery the same routine applied. Prior to and during the stripping operations, retention of maturing adults was necessary and a considerable degree of mortality in adult fish occurred. The loss thus occasioned in eggs wasted, varying from 13.4 per cent to 33.9 per cent of the total number of eggs contained in all females arriving at the counting weir, was noted, but in view of the fact that an appreciable portion of this loss may have been due to the unusual retention required by the investigation, a second set of calculations are given in which this loss is excluded.

During the stripping operations the loss in eggs occurring because of incomplete stripping varied from 2.1 per cent to 14.6 per cent, the average, based on eggs available for stripping, being 8.5 ± 1.4 per cent. During incubation of the eggs in the hatchery the losses in eggs varied from 4.4 per cent to 7.8 per cent for egg planting, and from 6.5 per cent to 18.1 per cent for fry liberation, the percentages being based on both total eggs available and eggs available for stripping. Taking into consideration all the operations involved in artificial propagation, it is found that the ultimate hatchery product represents approximately 60 per cent of the total eggs available, or 80 per cent of the eggs available for stripping. The difference of 20 per cent constitutes the loss in eggs due to female sockeye dying unspawned during retention and stripping operations.

In calculating the efficiency of artificial propagation it is found that no statistically significant difference prevails between fry liberation and egg planting,

the average for the three tests of the former being 2.66 per cent of total eggs available and 3.24 per cent of eggs available for stripping, while for the latter the averages were 2.27 and 3.08 per cent, respectively.

Finally, in comparing the efficiencies of natural and artificial propagation it is found that, when the variations between the several years' results are considered, there is no statistically significant difference apparent.

When consideration is given to the number of seaward-migrating sockeye produced from the three tests of fry liberation, it is found that the percentages of those migrants constitute, of the original fry liberations, 5.83, 3.85 and 2.81 respectively, the average being 4.16 ± 0.60. In other words, a mortality of approximately 96 per cent occurs during the year's residence of the young sockeye in the lake. This loss takes place after the artificial propagation activities have terminated and thus applies to artificially hatched and naturally reared sockeye alike.

ACKNOWLEDGEMENTS

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The Measurement of Firmness of Canned Salmon and other Semi-Rigid Bodies by the Dynamic Penetrometer Method

I. Experiments with a Multiple-Needle Penetrometer

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ABSTRACT

A multiple-needle penetrometer of the dynamic type is described. The results of experiments on canned salmon and a commercial shortening with a 12-needle penetration element show that single readings of depth of penetration can be obtained rapidly and are reasonably reliable in that for canned salmon the probable error of a single reading for an average depth of penetration of 10 mm. is of the order of 0.7 mm., while for a commercial shortening the corresponding probable error was only about one-half this value. Experimental evidence together with theoretical considerations, however, indicate that depth of penetration is not a valid measure of softness of canned salmon in the sense that the units of softness at different points along the scale are not equivalent.

INTRODUCTION

Firmness (or softness) often forms a valuable index of the quality of materials of a semi-rigid character. For example, firmness is of value in appraising the quality of raw and cooked meats, canned salmon and other canned fish products. It may also be employed in determining the quality of canned vegetables, canned fruits, shortenings, lard, greases, waxes, various petroleum products, asphalt and other industrial products. The firmness of a given material may, of itself, be a desirable quality characteristic or it may be valuable as a measure of a second quality characteristic, particularly when a high correlation exists between the firmness and the second quality characteristic. In the latter case the test for firmness may be of considerable value owing to the ease and rapidity with which the test can be carried out.

In measuring firmness by penetration methods, the degree of firmness is usually expressed in terms of one or the other of the following quantities: (a) the distance to which a "needle" loaded with a given weight penetrates the sample in a given time; (b) the weight that must be applied to the penetration element to cause the needle to penetrate a given distance. Since in method (b) the rate of penetration is ordinarily very low in comparison with that in (a), the two methods may, for convenience, be described as the *dynamic* and the *static* penetration methods respectively.

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The penetrometers commonly employed in testing greases, petrolatum, asphalt and cement (Vicot apparatus) apply the dynamic penetration method. This method has also been used by Sweetman and Lancaster (1931, 1932) and by Landreth (1929) for measuring the softness of cooked vegetables. Similarly, Tressler, Birdseye and Murray (1932) have applied this method in determining the relative tenderness of chilled and quick-frozen beef. The latter authors (*loc. cit.*) also devised a penetrometer of the static type, as did Bonney, Clifford and Lepper (1931), who developed an apparatus for testing the tenderness of canned vegetables and fruits.

As in the case of other quantitative tests, if measurements of firmness are to be scientifically and practically valuable, they should fulfil the following requirements: (1) the validity of the test should be high, that is, the degree to which the test measures that which it purports to measure should be high; (2) the reliability of the test, or the degree of accuracy or precision to which the test measures the character or property in question should be high; and (3) the test should be capable of being carried out rapidly. The outstanding advantage of the dynamic penetration method lies in the fact that it fulfils the third requirement, that is, the test can be carried out rapidly. This method also fulfils the second requirement satisfactorily. As will be shown below, this method furnishes measures of firmness or softness possessing reasonably low probable errors, when the apparatus is fitted with a multiple-needle penetration element. From the standpoint of the first criterion, however, the results obtained by both penetration methods appear to be faulty in that hitherto very little attention seems to have been paid to the effect of the velocity of penetration.

MULTIPLE-NEEDLE PENETROMETER

The penetrometer employed in these laboratories is an instrument manufactured by Messrs. Norman L. Armstrong and Company, Analytical Chemists, Vancouver, B.C. The instrument (figures 1 and 2) consists essentially of a carriage A bearing the three parts, the sample-holder B, the penetration element C and the scale D. The carriage can be moved up or down along the vertical support by means of the rack and pinion. The element C, which is made up of the needle-head and bar, moves vertically downward relative to the carriage when the clutch-release, E, is released. To take a reading the operator adjusts the sample-holder by moving the carriage, so that the sample-holder presses firmly against the sample. The clutch E is released and the needles allowed to penetrate for a given length of time. The depth of penetration is then read off on the scale opposite the upper end of the bar on the penetration element C. When testing canned salmon, the large circular opening in the bottom of the sample-holder enables the operator to adjust the penetration element so as to avoid penetration of the vertebral bone in the section of fish.

The needles employed in this instrument are chromium-plated steel needles, approximately 4.75 cm. in length, having points shaped in the form of the frustum of a cone. From measurements on six needles the average dimensions of the points were found to be as follows: Angle of cone, $A = 8.358°$, length of
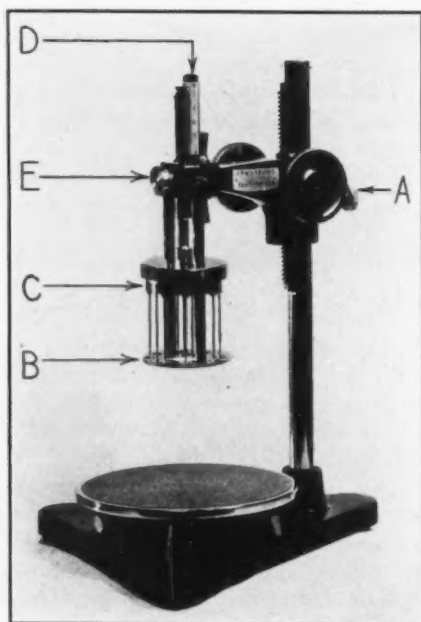
FIGURE 1. Multiple-needle penetrometer. A—carriage; B—sample-holder; C—penetration element; D—scale; E—clutch release.
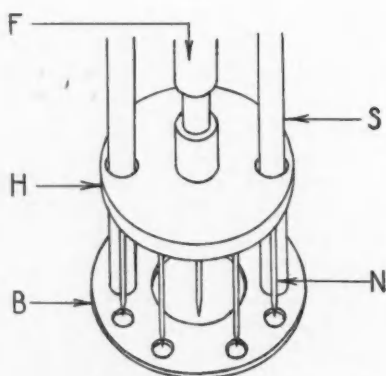

FIGURE 2. Details of sample-holder and penetration element. B—sample-holder; S—sample-holder support; N—needle; H—needle-head; F—bar attached to needle-head.

frustum = 0.860 cm., diameter of needle = 0.150 cm., tan $A/2 = 0.07307$, whence the average height of the small truncated portion = 0.166 cm.

Coefficient of Variation and Number of Needles

Preliminary experiments with the multiple-needle penetrometer were directed mainly towards increasing the reliability of a single reading. Preliminary tests with 3- and 5-needle penetration elements, with which the original instrument was supplied, indicated that the coefficient of variation (S/M) of a given number of readings on the same sample varied almost inversely as the number of needles in the penetration element (table I). The coefficient of variation corresponding to the 5-needle element was 0.274. Hence, if the law of decrease in coefficient of variation with increase in number of needles holds for a 12-needle element, the coefficient of variation, V_{12}, of readings for a 12-needle element on samples similar to that employed in the preceding experiments should be given by the equation

$$V_{12} : 0.274 = 5 : 12.$$

TABLE I. Variation in penetrometer readings obtained with 3- and 5-needle elements on the same sample of canned salmon. Time of penetration, 5 seconds. Total weight of penetration element, 250 grams.

No. of needles in penetration element	Readings (depth of penetration) (mm.)	Mean	Standard deviation	Coefficient of variation (S/M)
3	13.2, 20.0, 10.0, 9.2, 7.0, 17.0, 11.2, 12.2, 16.0, 25.5, 30.0, 17.0, 9.0..	15.2	6.52	0.429
5	12.0, 6.3, 7.8, 7.5, 9.0, 12.5, 8.0, 9.5, 11.0, 6.0, 5.2, 6.0, 11.0......	8.6	2.35	0.274

As will be seen from table II the value of V_{12} given by this equation, namely 0.114, is in good agreement with those obtained experimentally with a 12-needle element.

Since the data summarized in table II were obtained with an instrument that had been fitted with a sample-holder, they also give a general indication of the effect of the sample-holder on the coefficient of variation. Comparison with the data in table I shows that the sample-holder reduces still further the variation in the readings, owing probably to the fact that it smooths out irregularities in the surface of the sample and holds the latter firmly against the bottom of the can. The effect appears to increase with decrease in the number of needles in the penetration element.

The data listed under samples I and II, table II, are based on 13 readings only in each instance, and are therefore considerably affected by sampling fluctuations. More consistent values are shown under I and II combined, which

have been calculated by combining the data given under samples I and II. These results were combined as follows:

From elementary statistics (Yule 1927, pp. 134-135, 142) the combined mean is

$$m = \frac{n_1 m_1 + n_2 m_2}{n_1 + n_2}$$

where n_1, n_2, m_1, m_2 denote the numbers of measures and the means in the component distributions. But in this case $n_1 = n_2$, hence

(1)
$$m = \frac{m_1 + m_2}{2}.$$

Similarly for the standard deviation, since $s^2 = \sigma^2 + d^2$, where s^2 is the mean square deviation from the arbitrary origin d units distant from the mean, we may write

$$n\sigma^2 = n_1(\sigma_1^2 + d_1^2) + n_2(\sigma_2^2 + d_2^2),$$

TABLE II. Relationship between coefficient of variation and number of needles in penetration element. Time of penetration, 5 seconds. Total weight of penetration element, 250 grams. Number of readings for each element, 13. M=mean (mm.); S=standard deviation (mm.); V=coefficient of variation S/M.

	No. of needles			
	12	9	6	3
Sample I				
M	6.6	8.7	12.8	20.6
S	0.768	0.982	2.03	4.52
V	0.116	0.113	0.159	0.220
Sample II				
M	6.2	7.2	10.4	19.1
S	0.881	1.12	1.00	3.83
V	0.142	0.156	0.097	0.201
I and II combined				
M	6.4	7.95	11.6	19.85
S	0.850	1.295	2.002	4.256
V	0.133	0.163	0.173	0.214

in which $n = n_1 + n_2$, $m_1 - m = d_1$ and $m_2 - m = d_2$. Since, however, $n_1 = n_2$, then $d_1 = -d_2$ and therefore

$$n(\sigma^2 - d^2) = \frac{n}{2}(\sigma_1^2 + \sigma_2^2),$$

that is,

(2)
$$\sigma^2 = \frac{\sigma_1^2 + \sigma_2^2}{2} + d^2$$

where $d=d_1=d_2$ and σ is the standard deviation of the combined distribution. The application of equations (1) and (2) to the data listed under samples I and II gives the statistics shown under I and II combined.

Figure 3 shows graphically the relationships between the statistics m, σ and V of the combined distribution and the number of needles in the penetration element. In the case of the mean and the standard deviation, the relationships are definitely curvilinear. Within the interval 3 to 12 needles, however, the change in the coefficient of variation with increase in number of needles can be closely represented by means of a straight line. On fitting by the method of least squares the equation of this line is found to be, $V=0.234-0.00843N$, in which V denotes the coefficient of variation and N the number of needles in the penetration element. If the relationship is rectilinear, the best estimates of V from the above data are therefore as follows:
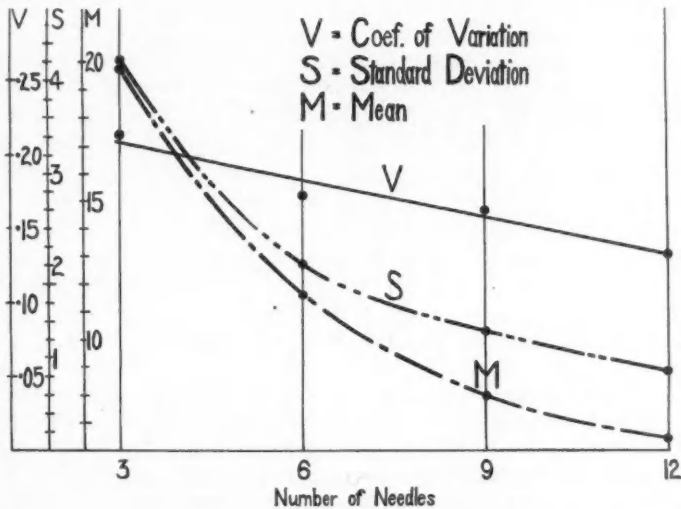

FIGURE 3. Curves showing mean, standard deviation and coefficient of variation as functions of number of needles in penetration element.

N	3	6	9	12
V	0.209	0.183	0.158	0.133

The preceding experiments, it should be noted, were carried out with a penetration element weighing 250 grams. Ordinarily, for routine testing of canned salmon in these laboratories a 200-gram weight is added to the element so that the total weight of the penetration element is 450 grams. The relationship between the coefficient of variation and the number of needles in the penetration element, however, is not appreciably altered by the addition of a 200-gram

weight as is evident from the following results derived from 13 readings on each of four different samples:

N	6	8	10	12
V	0.179	0.179	0.175	0.099
M	16.5	10.1	7.3	8.0

If a coefficient of variation of 0.10 is assumed for a 12-needle element for an average penetration of 10 mm., the probable error of a single reading is 0.67 mm. The average error in a single reading under these conditions is therefore of the order of 7 per cent. Furthermore, in the long run, the relative error would exceed 10 per cent only in 32 per cent of the readings and would exceed 20 per cent only in 5 per cent of the readings.

In the case of materials such as greases and waxes, that are more homogene-'ous than canned salmon, it seems reasonable to expect that variations in the

TABLE III. Variation in readings obtained with 12-needle element on a sample of commercial shortening containing occluded air. Total weight of element, 250 grams. Time of penetration, 5 seconds. Mean temperature of sample 16.5°C. M=mean; S=standard deviation; V=coefficient of variation.

Readings (depth of penetration) (mm.)	M	S	V
13.7, 13.3, 13.2, 13.8, 12.2, 12.8, 12.6, 13.1, 13.6	13.14	0.504	0.0384

readings will be less than those found for canned salmon. The results shown in table III indicate that the variation is, in fact, less for such materials, the variation in the case of the sample of commercial shortening being only about 0.05 as compared with about 0.10 for canned salmon.

The dynamic penetration method of measuring firmness or softness of certain classes of semi-rigid materials thus fulfils to a high degree the requirements of the criteria listed under (2) and (3). The test can be carried out rapidly, and, when the penetrometer is fitted with a multiple-needle element, the reliabilities of the measurements are of the order of those of many physical and chemical tests. There remains, therefore, the question of the validity of the test.

VALIDITY OF TEST FOR FIRMNESS

Without a clear definition of what is meant by the term "firmness" of a body, it is evidently impossible to determine the validity of the test, depth of penetration, as a measure of firmness. In the case of canned salmon the term "firmness" is usually associated in a general way with the ability of the sample to resist penetration, deformation and other physical tests which

alter the relative positions of the various parts of the sample. The firmness of a semi-rigid body of biological origin would thus appear to depend upon some intrinsic property of the fibres or cells forming the constituent parts of the body.

If this is so, then, when seasonal effects are eliminated, it seems reasonable to expect that the distribution of firmness in a large number of samples of canned salmon of the same species and packed under similar conditions would be normal or very nearly normal. That is to say, we should expect that for such a distribution the skewness and flatness would be those of a normal distribution, namely, $k = 0$ and $\beta_2 = 3.0$. The correctness of this view is reinforced by the fact that other quality characteristics of canned salmon, such as colour of the flesh and various characters of the fresh salmon, such as body weight and body length of the individual salmon, are distributed normally.

In the absence of any experimental evidence to the contrary, it appears justifiable, therefore, to apply the above principle in testing the validity of possible measures of firmness for canned salmon. Hence we shall say that a necessary condition for a valid measure of firmness for canned salmon is that the distribution of firmness in a large sample derived from salmon reasonably unaffected by seasonal changes shall be normal as regards the statistics k and β_2.

For practical grading purposes very considerable advantages result from a normally distributed measure of firmness for canned salmon. It should be noted, however, that such advantages are purely fortuitous and have no bearing on the question of the validity of the test. For non-normal distributions (Shewhart 1931, p. 181), the skewness $k_{\bar{x}}^-$ and flatness $\beta_{2\bar{x}}$ of the distribution of averages of samples of n are given in terms of the corresponding statistics of the universe by the equations

$$k_{\bar{x}}^- = \frac{k}{\sqrt{n}}, \quad \beta_2 = \frac{\beta_2 - 3}{n} + 3.$$

Hence it is possible from a knowledge of the skewness and flatness of the universe to calculate the size of sample required to reduce the values of these two statistics in the distribution of averages to approximately those for a normal distribution. When, however, the values of the statistics k and β_2 of the quality characteristic under investigation depart very appreciably from those for a normal universe, such a procedure not only involves additional expense for samples and cost of inspection, but is unsatisfactory, in that it is not possible, in most cases, to apply the extensive and valuable results that have been made available by modern statistical research, owing to the fact that the latter apply, for the most part, to normal universes.

TABLE IV. Distribution of depth of penetration of 1215 one-half-pound flat cans of sockeye salmon packed between July 15 and July 31, 1936. Mean $= 8.87$; standard deviation $= 1.81$. $k = \mu_3/\mu_2^{3/2} = 0.967$; $\beta_2 = \mu_4/\mu_2^2 = 5.51$.

X (depth of penetration) (mm.)	5	6	7	8	9	10	11	12
f (frequency)	7	73	192	275	274	216	89	46
X	13	14	15	16	17	18	19	20
f	22	10	5	2	2	1	—	1

As will be apparent from the data summarized in table IV, depth of penetration does not fulfil the condition for a valid test for firmness for canned salmon. These data, it will be observed, refer to samples of canned salmon packed within an interval of 16 days. Lack of constancy in the system of causes producing variation in depth of penetration on account of seasonal variation is therefore largely precluded. The penetration data, however, exhibit a pronounced positive skewness ($k = 0.967$) and are definitely leptokurtic ($\beta_2 = 5.51 > 3.0$). Divergencies from normality in the distribution of depth of penetration might, it is true, arise from differences in the proportions of fatty and muscle tissue in different sections of the fish, but such divergencies could hardly be expected to be of the order of those occurring in the data of table IV, inasmuch as variations in the proportions of fatty tissue in the section would not, in most instances, affect the resistance of more than one or two needles in the penetration element. As will be seen from table II, removal of 3 needles from the 12-needle element increased the average penetration by only about 1 to 1.5 units. Variation in depth of penetration arising from such an effect would not therefore account for the pronounced positive skewness in the data of table IV.

<div align="center">RELATIONSHIP BETWEEN DEPTH, TIME, AND WEIGHT</div>

The data given in table V show depth of penetration as a function of time of penetration corresponding to four different weights of the penetration element.

TABLE V. Depth of penetration (mm.) corresponding to different times
of penetration for samples of canned salmon.

Total weight (gm.)	Sample No.	5	10	20	30	Time (seconds) 60	90	120	150	180
250	1	5.0	5.1	5.2	5.4	5.7	5.8	5.9	5.9	6.0
	2	5.6	5.7	5.7	5.8	5.9	6.0	6.2	6.3	6.3
	3	4.9	5.2	5.4	5.5	5.7	5.8	6.0	6.3	6.3
	4	5.2	5.4	5.8	6.0	6.3	6.4	6.6	6.7	6.9
	M	5.18	5.35	5.52	5.68	5.90	6.00	6.18	6.30	6.38
450	1	8.1	8.4	8.7	8.8	9.3	9.6	9.6	9.8	9.9
	2	8.0	8.2	8.4	8.6	8.9	9.1	9.3	9.4	9.5
	3	8.8	9.0	9.4	9.6	10.0	10.4	10.4	10.6	10.8
	4	8.0	8.3	8.6	8.8	9.3	9.4	9.7	9.8	9.9
	M	8.22	8.48	8.78	8.95	9.38	9.62	9.75	9.90	10.02
650	1	9.3	9.8	10.2	10.6	11.2	11.9	12.3	12.9	13.3
	2	9.3	9.7	9.9	10.1	10.6	10.9	11.3	11.4	11.6
	3	9.1	9.5	9.7	10.0	10.4	10.8	11.1	11.3	11.5
	4	10.0	10.4	10.9	11.3	11.9	12.4	12.8	13.3	13.5
	M	9.42	9.85	10.18	10.50	11.02	11.50	11.88	12.22	12.48
850	1	14.0	14.3	15.0	15.4	16.3	17.2	17.6	18.0	18.3
	2	12.6	13.2	14.2	14.8	15.9	16.8	17.6	18.2	18.8
	3	12.8	13.4	14.5	15.1	16.3	17.4	18.1	18.7	19.3
	4	12.0	12.8	13.6	14.4	15.6	16.5	17.2	17.6	18.3
	M	12.85	13.42	14.32	14.92	16.02	16.98	17.62	18.12	18.68

<div align="center">M = mean</div>

To obtain the time-penetration data for any given sample, the load on the penetration element was first adjusted to the required weight and the sample-holder placed in position. The needles were then allowed to penetrate the sample, the time at which the clutch was released being recorded on a stopwatch. Readings of depth of penetration were obtained at the specified times as indicated by the stopwatch. To obtain the time-penetration data corresponding to the next load, the sample was turned through a small angle in the horizontal plane of the bench or else moved laterally so as to bring undisturbed portions of the sample directly below the needles. The average time-penetration data are shown graphically in figure 4. Similar data for a single sample of a commercial shortening and a penetration element weighing 850 grams are given in table VI.
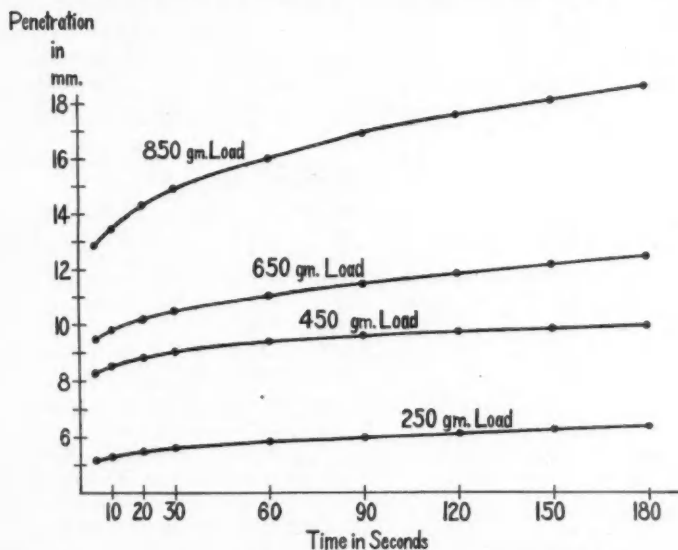

FIGURE 4. Curves showing depth of penetration as a function of time of penetration.

From figure 4 it will be observed that for an assigned value of t, say $t=5$, the depth of penetration, s, is not, as might be expected, directly proportional to the weight, w, of the penetration element. In fact, if values of s corresponding to a given value of t are plotted against w, it is found that s is connected with the latter variable by a relationship approximately described by the empirical equation

$$s = aw^3 - bw^2 + cw,$$

where a, b and c are constants for $t=$constant. The data of table VII indicate that a similar relationship exists in the case of the commercial shortening.

In the absence of any clear definition of the term "firmness" of canned salmon, it is of interest to express this property in terms of w connected with s by the relationship given in the previous paragraph for $t=5$ seconds. This

procedure is somewhat analogous to that followed in the static penetrometer method of measuring firmness, except that in the case of the data of table V, low values of w are associated with low values of s. Hence, in this instance, w would be a measure of the softness of the sample.

For $t=5$ seconds the relationship between the scales s and w derived from

TABLE VI. Depth of penetration as a function of time of penetration for a remelted sample of a commercial shortening. Total weight of penetration element, 850 grams. Temperature of sample, 19.5°C.

Time (seconds)	5	10	20	30	60
Penetration (mm.)	24.0	24.6	24.8	25.0	25.1
Time	90	120	150	180	....
Penetration	25.2	25.3	25.4	25.6	....

TABLE VII. Depth of penetration as a function of total weight of penetration element for remelted samples of a commercial shortening. Time of penetration, 5 seconds. Temperature of samples, 18.2°C.

Total weight (gm.)	Depth of penetration (mm.)			Mean depth (mm.)
250	6.1	6.3	6.6	6.33
350	8.0	7.8	8.1	7.97
450	10.4	9.8	9.6	9.93
550	11.0	11.3	11.9	11.40
650	14.2	14.0	14.0	14.07
750	17.0	16.8	17.0	16.93
850	19.6	20.5	19.6	19.90

TABLE VIII. Relationship between the scales, depth of penetration, s, and weight of penetration element, w, at $t=5$ seconds derived from the plotted data of table V.

Scale							
s	4.50	5.50	6.50	7.50	8.50	9.50	10.50
w	2.19	2.69	3.25	3.94	4.88	6.58	7.44
w (class mean)	2.44	2.97	3.60	4.41	5.73	7.01	
s	11.50	12.50	13.50	14.50	15.50	16.50	17.50
w	7.94	8.33	8.68	8.91	9.12	9.35	9.50
w (class mean)	7.69 8.14	8.50	8.80	9.01	9.23	9.43	
s	18.50	19.50	20.50				
w	9.62	9.75	9.81				
w (class mean)	9.56 9.69	9.78					

the data of table V is as shown in table VIII. On calculating the statistics for the distribution of softness of sockeye salmon given in table IV in the new scale w, it is found that the mean, $M=5.48$, the standard deviation, $S=1.64$, $k=0.226$ and $\beta_2=1.95$. The quantity, w, is therefore distinctly better than the depth of

penetration, s, as a measure of softness of canned salmon when considered in the light of the condition for a valid measure of firmness. The quantity w is also a more suitable measure of softness for practical purposes than s, the depth of penetration, since the skewness of the original distribution, when expressed in the scale w, is only 0.226 as compared with 0.967 for the scale s. When expressed in terms of w the distribution of softness, it is true, is slightly flatter than a normal distribution, but in a distribution of averages of samples of size 2 or 3 this value would rapidly approach the value for a normal distribution.

From the foregoing it seems clear that depth of penetration is not theoretically a valid measure of softness of canned salmon. The quantity, w, at $t=5$ seconds is a better measure of softness of canned salmon than depth of penetration. The quantity, w, however, fails to fulfil entirely the condition for a valid measure of softness or firmness for canned salmon. In a later paper in this series it will be shown that a more suitable measure of this property than either depth of penetration or weight of penetration element can be derived from a measure of the resistance of the sample to penetration.

ACKNOWLEDGEMENT

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The Adsorption of Vitamin A from Oils by Soaps formed *in situ*

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ABSTRACT

Soaps formed *in situ* removed vitamin A from oils by adsorption, which increased (a) with increase of the water : soap ratio up to 3,000; (b) inversely as the temperature of soap formation; (c) directly with the free fatty acid content of the oil. Percentage adsorption plotted against the original vitamin content of various oils gave a typical adsorption isotherm. Under comparable conditions, a preformed soap adsorbed 7%, a soap formed *in situ* 39%, of the vitamin. Of various fatty acid soaps investigated, oleates were most effective. The adsorbed vitamin can be recovered quantitatively.

It has long been known that during alkali refining of animal and vegetable oils by means of aqueous solutions of alkalies a certain amount of colouring matter is removed along with colloidally dispersed impurities. In some cases this removal of pigments by the soaps formed *in situ* appears to be of a selective nature. For instance, when salmon oil is neutralized with aqueous alkali solution under certain conditions, the red pigments are practically entirely removed while there is but little diminution in the amount of yellow pigment. These red pigments in salmon oil are carotinoids closely related to, if not identical with, astacin (Bailey 1937). β-carotene when dissolved in an animal or vegetable oil is also removed by soaps formed *in situ*, but not with the same ease as the astacin-like pigments. In view of the close structural relationship between the above pigments and vitamin A, it was considered likely that the latter substance might also be removed by such soaps. Unpublished experiments made some time ago by one of us indicated that this was true.

The recent demand for oils highly potent in vitamin A has led to much investigation regarding methods for the extraction of oils from the livers of such fish as the halibut, tuna, sword-fish, etc. As most of these methods involve the treatment of the livers or oil with an aqueous solution of an alkali somewhere in the process, it is of some economic importance to define under what conditions vitamin A may be removed from the oil, so that losses of the vitamin can be minimized. One method for avoiding such losses has already been noted (Brocklesby and Green 1937). The present communication gives the results of a study of the more important variable factors and offers proof that the

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removal of vitamin A from oils by soaps formed *in situ* is an adsorption phenomenon.

TECHNIQUE

The oils used throughout this work were halibut and dogfish liver oils of high quality. They were selected for their vitamin potency and low acid value, the latter never exceeding 0.50. Except in the experiments on the effects of various fatty acids, U.S.P. oleic acid was added to the oils in stated amounts. In all cases the aqueous alkali solution was added to the oil with mechanical mixing and in comparative experiments this technique was held constant throughout the run. The neutral free oil was separated either by gravity or by means of centrifuges, dried with sodium sulphate and filtered by suction. In no case was the free oil dissolved in any solvent during its isolation. Precautions were taken to protect the neutralized oils from oxidation whilst awaiting analysis. Usually they were analysed immediately after isolation.

Vitamin A assays were carried out in the main by the antimony trichloride reaction of Carr and Price (1926) as described by Drummond and Hilditch (1930, p. 31). A Rosenheim-Schuster type of Lovibond tintometer with a constant source of illumination was used during most of the work. Towards the end of the investigation a photo-electric colorimeter was available and this more precise instrument was used where indicated below. In one series of experiments the vitamin A was also estimated by the use of a Hilger vitameter-AC instrument. These values checked reasonably well with those obtained by the antimony trichloride reaction.

In determining the vitamin A potency by the antimony trichloride reaction the assay was made on the oil before the addition of oleic or other free fatty acids. Sufficient alkali was added to remove the natural and added free fatty acids. Thus the original and neutralized oils were examined under practically identical conditions and the interfering effects of free fatty acids noted by Norris and Church (1930) were eliminated. The oils were diluted with chloroform until 0.2 cc. of the solution plus 2.0 cc. of the antimony trichloride reagent gave a colour of between 5 and 6 Lovibond units in a 1-cm. cell. The number of such blue units in a gram of oil was then calculated and reported as the "blue value".

WATER: SOAP RATIOS

To nine parts by weight of a halibut liver oil with an initial blue value of 46,000 units per gram was added one part by weight of oleic acid. Ten-gram aliquots of this mixture were then neutralized with a predetermined amount of sodium hydroxide dissolved in various quantities of water. The alkali solutions and oils were heated to 95°C. before mixing and after mixing were allowed to cool to room temperature before separation of the oil by centrifuging. A similar series was made from another halibut liver oil with a slightly lower potency but in this case potassium hydroxide was used for neutralization. The results are indicated in figure 1. The effect of the soap concentration was very great, the more dilute the soap the greater the adsorption of vitamin A from the oil. At

dilutions of 1 in 1500 sodium oleate did not appear to have reached its maximum adsorption capacity. In the case of potassium oleate the loss by adsorption was very much greater and appeared to reach a maximum at a dilution of 1 in 1000. In a third similar experiment, using sodium hydroxide solutions for neutralization, the oils were assayed both by the antimony trichloride method and also by measuring the extinction coefficient at 328μ. The values were converted in each case to International units of vitamin A by conversion factors at present generally accepted. The data indicated in curves A and B of figure 2 are fairly concordant, the potency of the neutralized oils being in every case slightly
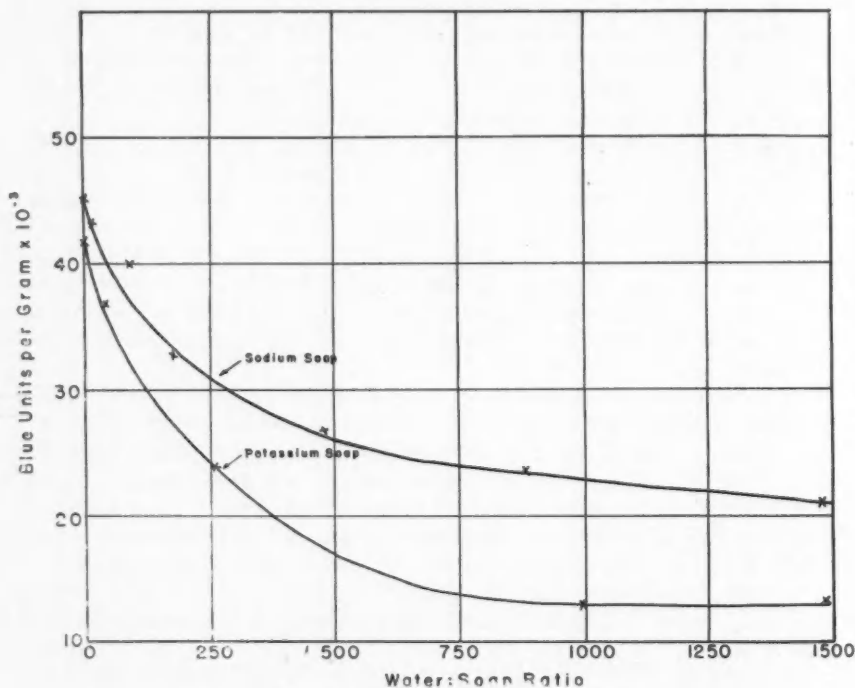

FIGURE 1. Effect of water: soap ratio on adsorption of vitamin A.

lower when calculated from the extinction coefficient than when calculated from the blue value. It will be noticed that the discrepancies between the two sets of values increase as the potencies of the neutralized oils decrease. We are of the opinion that the discrepancies are due to lack of strict applicability of the conversion factors which, it is well known, vary with the potency of the oil examined.

Many other experiments have been made in which the water : soap ratios had very low values. With ratios less than 25 the adsorption of the vitamin was relatively small and could be detected with certainty only in oils of high

potency.   At the other end of the range, mixtures with water : soap ratios as high as 3000 still showed considerable adsorptive power.   Since soaps lose their detergent properties when diluted, due to the formation of a true solution, this activity at such low concentrations was surprising.   If the water: soap ratio is increased to infinity some loss of vitamin A still takes place.   In one instance a neutral oil was shaken with one hundred times its volume of boiling water. The oil after drying showed no loss of vitamin A.   But the same oil containing
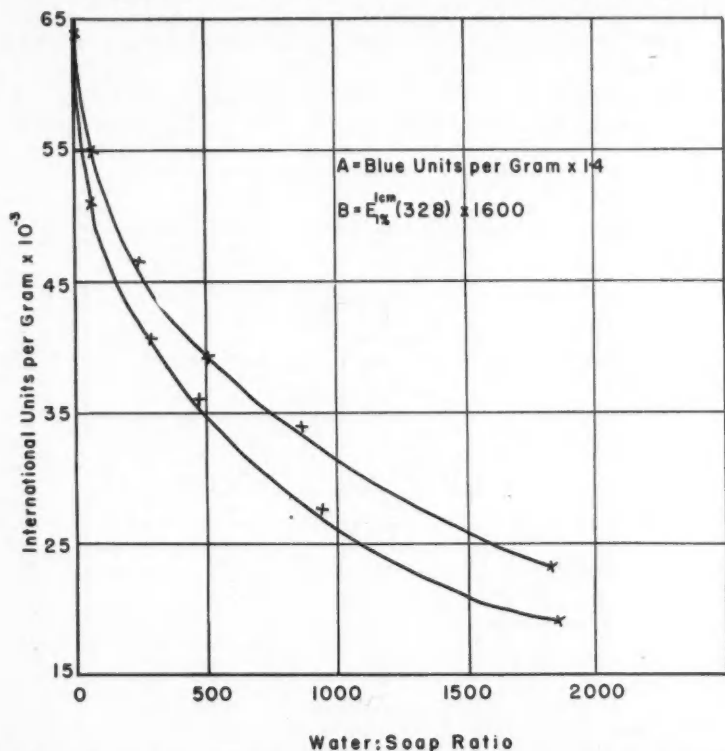

FIGURE 2.   Comparison of adsorption losses at various water:soap ratios using antimony trichloride reaction and extinction coefficient.

10 per cent of oleic acid treated in the same way lost approximately 5 per cent of its vitamin potency.   In the first instance the emulsified oil cleared up and separated immediately but in the second the separation was slower and the water layer decidedly murky, indicating that a more persistent emulsion had been formed, probably through interfacial orientation of the free fatty acid molecules.

EFFECT OF TEMPERATURE

A halibut liver oil having a blue value of 55,000 units per gram was diluted with oleic acid so that the total free fatty acid content was 10 per cent.   Ten-gram aliquots were then exactly neutralized with sodium hydroxide dissolved in

500 cc. of water. The oils and alkalies were heated to various temperatures before mixing. After neutralization the emulsions were separated immediately by centrifuging but no attempt was made to keep the mixtures at their respective temperatures during this latter process. The neutral clear oils were then dried with sodium sulphate, filtered and assayed. The data are indicated in figure 3 and show that the adsorption of vitamin A decreased as the temperature increased. The bulk of the soap formed *in situ* was sodium oleate since the natural free fatty acids in the oil amounted to only 0.3 per cent. Sodium oleate tends to form true solutions as the temperature is increased and thus the capacity
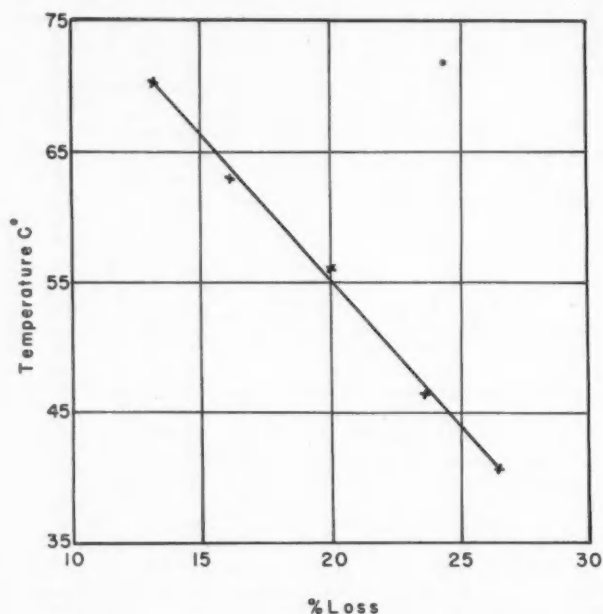

FIGURE 3. Effect of temperature of soap formation on adsorption of vitamin A from oils.

to adsorb decreases. These data support the conclusion that the loss of vitamin A through soaps formed *in situ* is not due primarily to a chemical oxidation but to the colloidal nature of the soap formed.

FREE FATTY ACID CONTENT

To weighed samples of a neutral halibut liver oil were added varying amounts of oleic acid. These samples were then neutralized at 95°C. with the predetermined amount of sodium hydroxide dissolved in such an amount of water that the resulting water: soap ratio was 500 in every case. The neutral oils were recovered immediately after neutralization. The data are indicated in figure 4, plotted as per cent loss of vitamin A against free fatty acid content

and show that the loss was proportional to the amount of free fatty acids present or, in other words, to the amount of soap formed. It will be noticed that the curve does not meet the X-axis at zero per cent loss. Although the data are not precise enough to indicate exactly where the curve should meet the X-axis we have found that even though an oil be neutral, alkali refining will always show a small loss, usually detectable only in highly potent oils.

VITAMIN POTENCY OF ORIGINAL OIL

Further proof that the removal of vitamin A from an oil during neutralization is an adsorption process was found in plotting the loss of vitamin against the original potencies of a number of oils. These were made up from two neutral
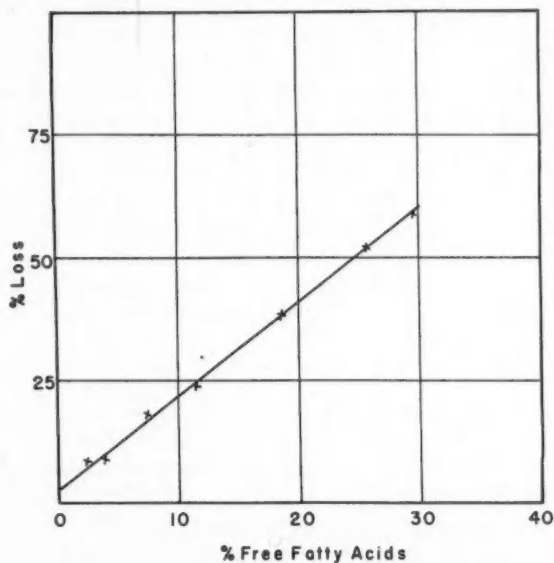

FIGURE 4.   Effect of free fatty acids on adsorption of vitamin A from oils when neutralized with aqueous alkali.

samples, a high and a low potent oil. They were mixed in various proportions and the blue values of the mixtures determined. To the mixtures was added sufficient oleic acid to make the free fatty acid content 10 per cent. These oils were then neutralized at 95°C. with sodium hydroxide of such strength that the resulting water: soap ratio was in each case exactly 500. Recovery of the neutralized oils was accomplished as usual with the centrifuge. The data are indicated in figure 5 by plotting the blue units per gram of the original oils against their respective percentage losses after neutralization. The curve shows the essential characteristic form of the adsorption isotherm. If the logarithm of the vitamin A concentration of the original oils is plotted against the logarithm of the vitamin A adsorbed a straight line curve results. From a

practical standpoint the curve indicates that under otherwise comparable conditions the loss to be expected in a neutralization is not directly proportional to the potency of the oil. The curve also indicates, of course, that for any one set of conditions regarding temperature, amount and concentration of soap present, there is a saturation point beyond which no more vitamin A will be adsorbed.
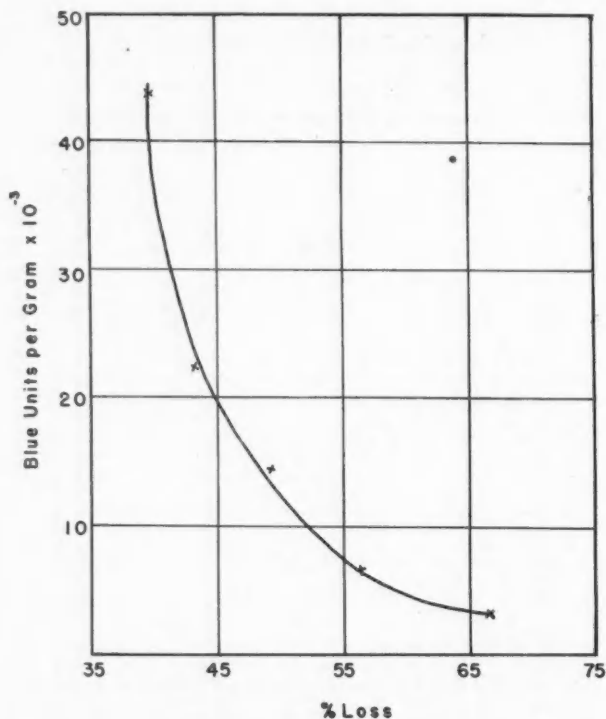

FIGURE 5. Relation between vitamin potency of original oil and loss by adsorption.

EFFECT OF VARIOUS FREE FATTY ACIDS

The bulk of the work so far described was carried out with sodium or potassium oleates. In actual practice a variety of fatty acids is met with dependent upon the nature of the oil or fat being refined or isolated. We have therefore investigated the effect of eight naturally occurring fatty acids when added individually to a neutral halibut liver oil. Each of the eight mixtures contained 10 per cent of the particular fatty acid and each was neutralized with sufficient potassium hydroxide dissolved in water to make the water: soap ratio equal to 500. Unfortunately, the neutralizations could not all be made at the same temperature in view of the great differences in the solubilities of the soaps. The data given in table I show that the losses in vitamin A by adsorption follow fairly well the known properties of the particular soap. In the homologous

TABLE I. Effect of various fatty acids on adsorption of vitamin A.

Acid	Temperature of neutralization (°C.)	Vitamin A lost in neutral oil (per cent)
Caprylic	18	0
Capric	18	0
Lauric	18	2.8
Myristic	60	6.5
Palmitic	60	60.0
Stearic	60	7.0
Oleic	18	71.3
Linolenic	18	45.7

series of fatty acids the detergent properties of the alkali salts commence with those of caprylic acid and become very pronounced with those of lauric acid. In the present case, at a temperature lower than 60°C. potassium myristate would probably have shown a greater adsorbent power, but on the other hand at such lower temperatures potassium palmitate and stearate would have been much less soluble. At 60°C. the myristate did not show very pronounced emulsifying properties whilst the stearate was thrown partly out of solution. The alkali salts of oleic acid are recognized as being superior to any others in detergent properties, and the present data show that the adsorbing capacity of its potassium salt for vitamin A is greater than any of the others investigated. It must be remembered, however, that under suitable conditions it may be possible to increase the vitamin adsorption of any of these soaps.

PREFORMED SOAPS VERSUS SOAPS FORMED *IN SITU*

The high surface activity of soaps formed *in situ* (nascent) has been known for a long time but it was only recently that this property was applied in an industrial way. Some properties and industrial applications of nascent soaps are discussed by Rayner (1934). It had been our experience that a preformed soap never removed as much pigment from an oil as one formed *in situ* in the oil. This has proved to be the case also in the adsorption of vitamin A. The following example will illustrate the striking difference obtained by the two methods of soap formation. To a 10-gram sample of neutral halibut liver oil with a blue value of 45,000 units per gram was added 1 gram of oleic acid. The oil was then neutralized at 95°C. with sufficient sodium hydroxide solution dissolved in water to give a resulting water:soap ratio of 500. The neutralized oil was recovered by centrifuging and after drying was found to have a blue value of 27,500 blue units per gram. In the second case, 1 gram of oleic acid was neutralized with the same amount and concentration of alkali at 95°C. and the hot soap solution added to 10 grams of the same halibut liver oil. The mixture was shaken in the same manner as in the previous case and the neutral oil recovered. After drying it was found to have a blue value of 42,000 units per gram. The soap formed *in situ* adsorbed approximately 39 per cent of the vitamin whilst the preformed soap adsorbed but 7 per cent.

RECOVERY OF ADSORBED VITAMIN A

The removal of vitamin A from oils by adsorption on soaps formed *in situ* did not necessarily indicate that the adsorbed vitamin could be recovered. We were interested in ascertaining whether or not any loss through oxidation took place subsequent to the adsorption. A large number of experiments in which the free and adsorbed oil (and vitamin) were taken up in various solvents and recovered by distillation of the solvents *in vacuo* all gave yields ranging from 50 to 70 per cent of the total number of units of vitamin A present in the original amount of oil used. It was found that the loss took place through oxidation during recovery with the various solvents used. Finally a method was adopted that showed conclusively that *during the adsorption process* no vitamin A was lost through oxidation. Briefly the method was as follows: a halibut liver oil containing 10 per cent oleic acid was neutralized with sodium hydroxide at a temperature of 95°C. and of such dilution that the resulting water:soap ratio was 500. The free neutral oil was separated mechanically from the emulsified oil and soap mixture. Both portions were shaken with chloroform after the addition of sufficient barium chloride to destroy the colloidal properties of the sodium soap. Extractions were continued until test portions no longer gave any colour with antimony trichloride. The two solutions were washed with cold water, dried and made up to definite volume. Aliquots were then tested quantitatively by the antimony trichloride reaction when it was found that practically 100 per cent of the original vitamin A was accounted for in the two solutions. For example, in one case 4 grams of an oil with a potency of 58,000 blue units per gram were diluted with 0.5 gm. of oleic acid and neutralized as above. The chloroform solution of the recovered neutral oil contained 73,000 blue units whilst the chloroform washings of the soap mixture contained 158,000 units. Of the 232,000 units of vitamin A in the original oil, 231,000 units were recovered.

During the course of this latter work it was noticed that if the aqueous soap solution containing the adsorbed and emulsified vitamin and oil was treated with a soluble alkaline earth metal the precipitated soap carried with it all the vitamin A. The latter could then be removed from the precipitated soap by washing with a suitable solvent. It is, however, extremely difficult to remove the adsorbed vitamin A from the sodium or potassium soaps with any of the fat solvents immiscible with water. Those solvents that are miscible allow the dissolution of the vitamins to take place by destroying the colloidal properties of the soap either by forming a true solution or by precipitation of the soap from solution.

In conclusion it is of some interest to record a few observations which have a bearing on the mechanism of the adsorption. When a deeply pigmented salmon oil is neutralized under suitable conditions, the neutral oil which separates is yellow in colour but the aqueous soap layer is of a rose pink colour. If this soap is centrifuged at very high speeds emulsified oil particles are separated and the soap solution becomes quite clear and optically blank at magnifications of 400 diameters. The oil separated by centrifuging is still lacking in the red

pigment but the clear soap solution is red. If this is passed through a Seitz filter the red colour is still retained by the solution. A similar thing occurs with vitamin A. If the lower layer of emulsified oil and soap solution is passed through a Seitz filter a sparkling clear liquid is obtained. When this is shaken with any fat solvent only a very small amount of vitamin A is removed. When the soap is put into true solution by the addition of alcohol, vitamin A may then be recovered in a very concentrated form by shaking with a fat solvent such as chloroform. Quantitative tests show that the sparkling clear soap solution contains a very small amount of oil. Whether or not the vitamin A can be desorbed free of this small trace of oil is a problem which is at present under investigation.

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Mortality Trend Among Young Sockeye Salmon (*Oncorhynchus nerka*) During Various Stages of Lake Residence

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ABSTRACT

Young sockeye salmon, marked and liberated into Cultus lake, B.C., at intervals during the year were counted as seaward migrants. A linear relationship between time in the lake and percentage survival was found for periods of from 9.5 to 3.5 months, and from this the trend in percentage loss is computed. Mortality is found to be heavy during the first few months— approximately 65.4 per cent in the first 2.5 months—decreasing, however, as the year advances and the young sockeye increase in size.

Investigations of the efficiency of the artificial propagation of sockeye (Foerster 1936, 1938) at Cultus lake, British Columbia, have indicated that for three tests of sockeye salmon fry distribution, the resulting seaward migrants constituted 5.83 per cent in 1926, 3.85 per cent in 1929 and 2.81 per cent in 1932 of the total number of fry liberated, the average being 4.16 per cent. In other words, a mortality of not less than 94 per cent of fry liberated in the lake occurred during the year's residence.

In view of the vital significance of this extremely high mortality occurring during the sockeye's lacustrine stage, an endeavour was made to determine at what period or periods of lake residence the greatest losses occurred.

METHODS

To determine the natural mortality trend in the lake, young sockeye were reared in retaining ponds adjacent to the lake and, at certain periods during the year, known numbers were liberated. For each group liberated a distinctive mark was applied by removing certain fins. At the time of seaward migration of the normal lake population of sockeye, when all of the downstream-migrating individuals were trapped and enumerated, the marked fish were segregated and counted. The bulk of these latter were recovered in the migration of the following spring as yearlings but a number were also obtained in the second spring following, as two-year-old individuals. By totalling the recoveries of each series of marked fish, the percentage survival from each liberation into the lake was obtained.

Three experiments were undertaken, in the years 1930-31, 1932-33 and

1934-35 (see table on page 187). In 1930, Costiasis occurred, causing losses, between June 2 and October 6, of 90 per cent of the original pond population. In 1932 Octomitiasis appeared with equally heavy losses. Mortality occurred chiefly during August and September, when water temperatures were high and the ponds possibly overcrowded through a desire to have as many experimental fish as possible available for marking and liberation. To what extent the young fish liberated in 1930 and 1932 prior to the visible onset of the diseases had become infected and subsequently suffered abnormal mortality in the lake remains unknown, but the final liberations of fish that had either recovered from the disease or had not been affected appeared to be satisfactory. These have been used in confirming the trends indicated by the data of the third experiment. In the 1934-35 test, no disease occurred and pond mortality was slight.

Of those factors introduced into the experiments and pertaining to the experimental fish which would obviously not apply to normal naturally-spawned and naturally-reared sockeye, only two are considered of major importance. These consist of (1) the marking operation and the consequent handling of the fish, and (2) the increase in growth or size resulting from the artificial feeding in the ponds.

With respect to the former, the marking operations, it was found that among groups of marked fish held in a small pond for a period of two weeks mortality was less than 0.2 per cent and did not vary according to the mark used. Furthermore the data obtained from the experiments do not show any irregular variation in the lake mortality irrespective of whether one, or two fins were amputated, or whether the fish were 49 mm. or 112 mm. in length. It would appear that the marking operations and the consequent handling did not seriously affect the lake mortality trend discovered.

Concerning the relative sizes of the experimental fishes in comparison with the normal lake resident sockeye, there was no way in which the growth rate of the latter during the first year could be traced and conformed to in the pond-reared individuals. The general concern was to avoid heavy mortality in the ponds and to produce healthy, virile fish.

There are tabulated below the lengths of the experimental sockeye at time of liberation and of migration.

Date of liberation	Size at liberation				Size as seaward migrants			
	Number in sample	Length	P.E.	P.E.$_m$	Number in sample	Length	P.E.	P.E.$_m$
July 13-18, 1934	50	4.9 cm.	0.31	0.044	202	8.7 cm.	0.68	0.036
Sept. 12-14, 1934	50	7.8 "	0.49	0.055	296	9.4 "	0.40	0.024
Nov. 14-21, 1934	50	9.4 "	0.94	0.097	279	10.0 "	0.50	0.030
Jan. 14-17, 1935	50	9.7 "	0.56	0.057	138	10.6 "	0.53	0.045
Mar. 11-18, 1935	49	11.2 "	0.99	0.093	135	10.8 "	0.66	0.06

From a sample of 1,503 normal seaward migrating sockeye the average length for the 1935 migration was found to be 9.1 ± 0.0097 cm.

Since the last three experimental groups liberated were all larger at time of

liberation than the normal lake sockeye at time of migration, the pond fish were probably of greater size than the normal ones after about November 1, or possibly earlier. The increased growth, in comparison with the lake sockeye, may have resulted in a greater survival to migrant stage, but it seems to be discounted by the evidence, shown below, of a linear relationship in per cent migration throughout and the fact that the first two groups did not differ greatly in size from the normal sockeye at time of migration.

On the contrary, there is evidence to indicate that the larger size of the latest groups of liberated fish, rather than weighting the data favourably by an abnormally high survival, affected them in opposite fashion by contributing to the non-migration of individuals. It has recently been observed by Ricker (1938) and referred to in a previous paper (Foerster 1937a) that in some years at least, if not in every year, there occur in Cultus lake certain populations of sockeye the individuals of which remain in the lake and mature. Specimens have been taken in small-mesh gill nets and have been found maturing in their second, third or fourth years. These sockeye show distinct differences in colour, size, habits and sex ratio to the common landlocked sockeye (*O. nerka kennerlyi*) and Ricker has given to them the name "residual" sockeye.

These "residual" fish, according to Ricker, are believed to result, in part, from normal young sockeye of anadromous stock which have experienced unusually rapid growth during their first or second years and do not respond to the migratory stimuli in the spring.

Because of their large size at time of liberation, some of the larger individuals in the experimental groups may have reacted in similar manner to lake conditions, may have become residuals and thus not been encountered in the seaward migrations. Fish of the last two or three groups may have been so affected. Supporting evidence is provided by the data for the final liberation in 1934-35 which shows that, whereas the mean length at time of liberation was 11.2 ± 0.093 cm., that for the migrating individuals of this group was 10.8 ± 0.006 cm. This statistically significant decrease may well indicate that only the smaller individuals entered upon migration and that the largest continued to reside in the lake as "residuals". For the final liberation from the 1932-33 experiment a similar situation was indicated by the capture in October 1934 in the lake of a marked individual from this group. It was then in its third year and was maturing.

In analyzing the data from the experiments, therefore, the possible deviation from a natural situation, as resulting from firstly, the marking, and secondly, the increased size of the liberated fish, has been considered as tending in the direction of providing fewer recoveries, particularly with respect to the last or last two groups in each experiment. The trend, as calculated from the data, is believed to be a general picture of normally prevailing conditions, although the mortality, or conversely the survival values at individual periods, may be to some extent altered. The extrapolated survival rates shown for the first few months of lake residence are possibly higher than would prevail under natural conditions. Conversely the rates for the last few months' residence, as depicted by the last two or three liberations, are probably lower than normal.

RESULTS

In the table below are recorded the data for each of the three experiments. The time of pond retention is estimated from date of transfers to ponds to mean date of liberation. The period of lake residence is estimated from mean time of liberation to mean time of migration. As explained in the preceding section, those for the third year have been principally utilized but the results of the final liberations in the two earlier tests have also been used. If the remainder of the data in these first two experiments are of any value it would appear to be in the nature of indicating the after-effects of disease upon fish liberated from ponds in which infection had occurred.

Dates of liberation	Period of retention (months)	Fins removed	Sockeye liberated	Sockeye recovered as migrants 1st yr.	2nd yr.	Total	Per cent of liberation	Period of lake residence (months)
1930-31								
October 6	4.	Adipose	41,144	4,362	none	4,362	10.6	6.5
March 16	9.5	Two pelvics	15,922	10,051	none	10,051	63.1	1.5
1932-33								
July 18-27	2.5	Adipose	94,483	4,211	41	4,252	4.5	9.0
Aug. 16-23	3.5	Two pelvics	93,756	640	13	653	0.7	8.0
Sept.15-19	4.5	Right pelvic	51,651	115	3	118	0.2	7.0
March 29 - April 2	11.0	Two pelvics and adipose	31,050	20,575	2	20,577	66.3	1.0
1934-35								
July 13-18	2.0	Two pelvics	25,000	5,461	19	5,480	21.9	9.5
Sept.12-14	4.0	Adipose	25,000	8,205	1	8,206	32.8	7.5
Nov. 14-21	6.0	Left pelvic	40,000	17,833	4	18,837	44.6	5.5
Jan. 14-17	8.0	Right pelvic	40,000	21,870	2	21,872	54.7	3.5
Mar. 11-18	10.0	Two pelvics and adipose	53,375	17,554	2	17,556	32.9	1.5

In figure 1 the percentage recoveries (as given in the table) for the several groups liberated at stated times during 1934-35 are plotted against the period of residence in the lake, the latter being calculated from the mean time of liberations to the mean time of migration. For the period extending from 9.5 to 3.5 months in the lake, roughly from mid-July to mid-January, a straight line relationship is found to be the best expression. The last point, representing 1.5 months' residence in the lake, falls low, 32.9 per cent survival, probably the result, as outlined in the preceding section, of a non-migration of some of the individuals due to their unusually large size at time of liberation. The probably more normal position of this point is indicated by the data of the first two experiments which showed for the final liberations of 1930-31 and 1932-33, percentages of survival of 63.1 for one and one half months' lake residence and 66.3 for one month respectively. If the line joining the four points from 9.5 to 3.5 months' lake residence be extrapolated to one month in the lake, a survival of approximately 68.5 per cent would be obtained, a value which closely approximates those of the two former years.

From three liberations of free-swimming fry into Cultus lake, as noted in the introduction, the percentage survival ranged from 2.81 to 5.83 per cent, with a mean at 4.16 per cent. If, in figure 1, the trend of survival from the pond-reared fish is projected to include this fry survival, a slightly steeper slope of the line is noted. If, on the other hand, the line joining the four points
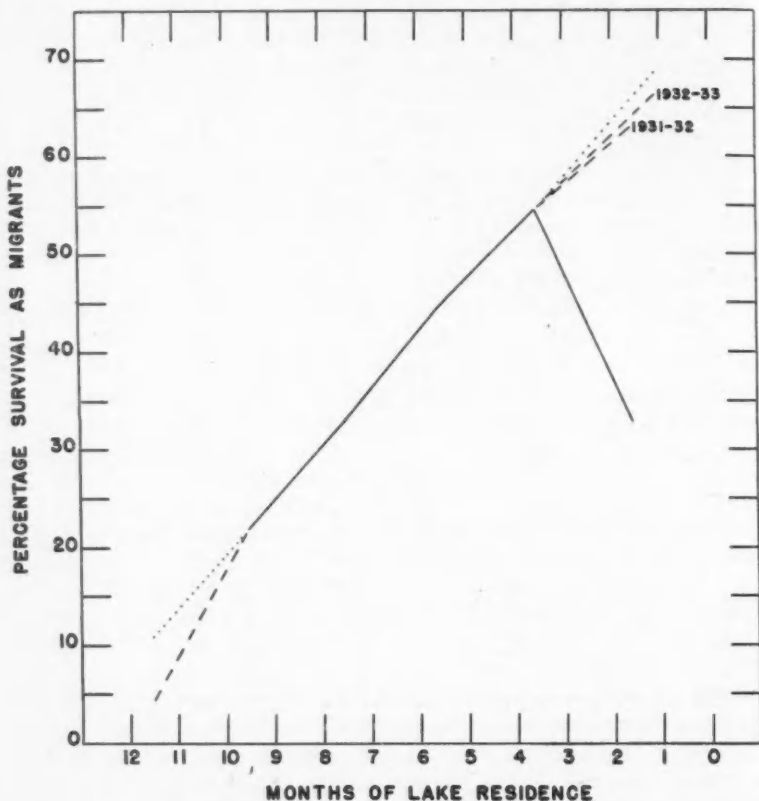

FIGURE 1. Plot of the five percentage recovery values obtained for the indicated periods of lake residence in 1934-35. The relation of the mean percentage survival from three fry liberations (4.16%) and of the comparable percentage survival records for the final liberations in the 1930-31 and 1932-33 experiments is shown (broken line), together with the probable values to be obtained by extrapolating the line indicated by the four points in 1934-35 (dotted line).

obtained from 9.5 to 3.5 months' lake residence be extrapolated to the 11.5 month period, it is found that for the latter period the migration percentage would approximate 10.5 to 11 per cent. To what extent the increased mortality indicated by the actual percentage survival from fry liberations is a normal condition, or to what degree it is due to added losses inherent in the handling of fry at time of liberation, is not known but it is probable that some of the increase thus results.

The trend in the mortality among young sockeye in the lake may perhaps be more clearly portrayed by calculating the number of sockeye that must be in the lake at different periods of the year in order to provide for 100 migrants. From the least square treatment of the percentage survival values of the four groups in 1934-35, the constants a and b in the equation $y = a + bx$ were calculated as 74.31 and -5.51 respectively, where y represents the percentage survival values obtained, x the number of months in the lake, a the percentage of yearlings which actually migrate, and b the rate of change of the percentage of young sockeye liberated in the lake which will migrate, i.e., the slope of the line in figure 1.

By utilizing the reciprocal of the above equation it is possible to calculate the number of fry or fingerling sockeye which must be available in the lake at any time (x months) in order to produce a given number of seaward migrants. The numbers required to produce 100 migrants are given below. Only for the period from 9.5 to 3.5 months, however, are there observational data. The losses per month, in numbers and percentage, are also shown.

Months in lake	Number to be liberated	Loss per month	Percentage of those surviving	Loss per month of original liberation
11.5	914	184*	20.1*	20.1*
11.0	730	209	28.6	22.9
10.0	521	116	22.3	12.7
9.0	405	74	18.3	8.1
8.0	331	51	15.4	5.6
7.0	280	38	13.6	4.2
6.0	242	28	11.6	3.1
5.0	214	23	10.7	2.5
4.0	191	18	9.4	2.0
3.0	173	15	8.7	1.6
2.0	158	13	8.2	1.4
1.0	145	10	6.9	1.1
0.0	135			

*half-month only.

Since observations at Cultus lake indicate that free-swimming fry rarely occur until May, a period of 11.5 months is taken as the approximate period these fish are resident in the lake. The above tabulation suggests that for each 100 seaward migrants produced, 914 fry must be available. The data relative to fry distributions in other years, as noted above, would require a much larger initial stock. As the period of residence in the lake grows less the degree of loss decreases until at 0.0 months, just prior to migration, 135 yearlings are required to provide 100 migrants. Even supposing the extrapolation to be valid it must be remembered that because of the unusually large size of the later experimental fish there was probably among them a larger number of non-migrants (residuals) than among a normal population. Probably, therefore, fewer than 135 normal sockeye would suffice to produce 100 migrants.

By dividing each of the items in column two of the tabulation above by 914 the percentage survival trend from time of liberation in the lake to time of seaward migration may be calculated and is plotted in figure 2. At the end of 11.5 months in the lake 14.7 per cent survive. The actual migrants, however, represent but 100/914 or 10.94 per cent of the liberated fry or 74 per cent of the 14.7 per cent potential migrants in the lake. If the line in figure 1 be extrapolated to zero months in the lake it will be found to bisect the ordinate at 74 per cent.

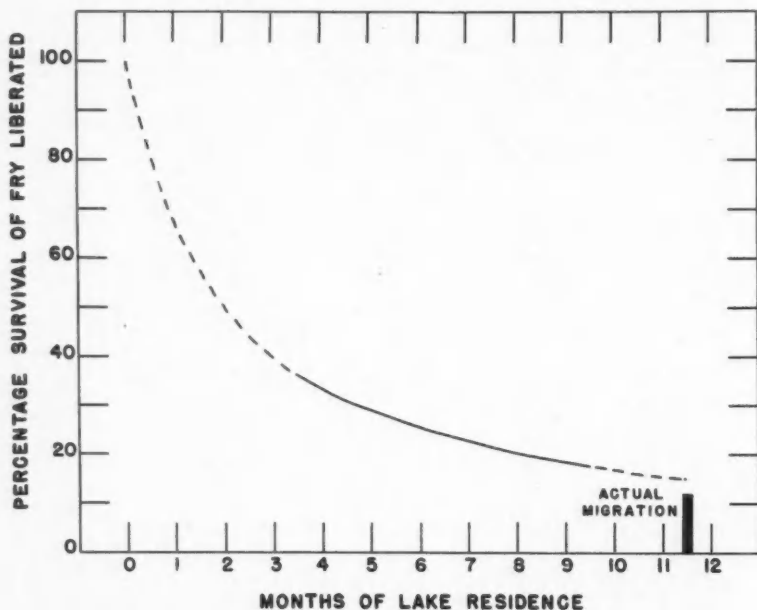Of particular significance is the fact, as shown in the text above and in

FIGURE 2. Curve indicating the percentage survival trend of young sockeye in Cultus lake from the time of liberation to time of seaward migration. At 11.5 months' lake residence, i.e., time of migration, 14.7 per cent of the fry liberated survived but only 74 per cent migrated or 10.9 per cent of the original fry liberation.

figure 2, that the mortality trend in the lake is high during the first few months (65.4 per cent in the first 2.5 months). Thereafter it rapidly decreases. During the fry and early fingerling stages, therefore, the major losses occur, prevailing during the months of June to August. If, as intimated in another paper (Foerster 1937b) a large fraction of this sockeye mortality is due to predation by predatory fishes—squawfish (*Ptychocheilus oregonensis*), char (*Salvelinus malma*), trout (*Salmo ciarkii*), etc., the period of greatest loss would appear to be the summer months. Observations likewise indicate that heavy predation occurs just prior to and at the time of seaward migration. This may in part

account for the discrepancy between data for sockeye available in the lake for migration and those which actually migrate.

The data obtained from those experiments may not accurately indicate the real extent of mortality occurring in all phases of the lake residence of young sockeye salmon. It is felt that the losses shown are probably too low for the earliest liberations (9 to 11.5 months' lake residence) and too great for the latest (last two to three months in the lake). Nevertheless they reveal a general trend which is of importance in considering measures of conservation which may apply to the more successful production of sockeye from available stocks.

SUMMARY

From the data provided by a series of liberations into Cultus lake of distinctively marked young sockeye, all hatched at the same time and retained for varying periods in retaining ponds, it has been possible to portray the relative rate of mortality occurring in the lake at different periods of the year. A linear relationship between period of residence in the lake and percentage survival is found to apply from 9.5 to 3.5 months in the lake. The actual percentage loss in numbers of fry liberated is heavy during the first few months of lake residence—approximately 65.4 per cent occurred in the first 2.5 months—but decreases as the year advances and the young sockeye increase in size.

ACKNOWLEDGEMENTS

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"Residual" and kokanee salmon in Cultus lake*

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ABSTRACT

The sockeye salmon in Cultus lake are of three kinds: (1) the normal anadromous stock, (2) a "residual" group, not anadromous, but largely or perhaps wholly the progeny of anadromous fish, and (3) a stock of non-anadromous kokanee, which have no known connection with the anadromous or residual fish at the present time. Morphological and physiological peculiarities distinguish the three kinds of sockeye at maturity. In the segregation of the progeny of a spawning of anadromous fish into migrant and residual groups, rate of growth and sex play a determining role.

INTRODUCTION

In the course of the study of the lacustrine life of young sockeye in Cultus lake, B.C., it has been discovered that there exist in the lake *Oncorhynchus nerka* of three different types. These are the anadromous sockeye, the kokanee or "land-locked" sockeye, and a third type, for which the name "residual" sockeye is proposed. The anadromous sockeye are distinguished by the fact that the young migrate to sea after one, or less often two years of life in the lake, and return again in the second or third year following. Sockeye of the second type are non-migratory, spending their whole life in fresh water. In their bright red breeding coloration these fish closely resemble the kokanee (sometimes separated as *O. n. kennerlyi* Suckley) which are known to inhabit many lakes of the Pacific coast drainage. Sockeye of the third type are also non-migratory, but differ markedly from the kokanee in breeding colour, time of spawning and certain other characters. The name "residual" sockeye is proposed for them because the evidence shows that they are, at least in large part, the progeny of anadromous parents.

This paper is devoted to a description of the residual sockeye and the kokanee of the lake, with particular reference to those features by which they may be distinguished. Although residual sockeye are not entirely new to scientific literature, there has never yet been any suggestion that they were in any way separable from kokanee. Attention is given also to those characters which separate the young residual from young anadromous sockeye, in order to obtain a better understanding of the factors which influence the migratory habit.

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*Paper No. 5 of a series: Factors affecting the behaviour and survival of sockeye salmon (*Oncorhynchus nerka* Walbaum) during their lacustrine existence in Cultus lake, British Columbia.

In spite of the emphasis that such differences will receive, the reader should not forget the fundamental similarity between all three forms of sockeye. The anadromous and residual sockeye represent merely two different ways of life adopted by different members of a single population. Kokanee, although in Cultus lake distinct from the other forms by reason of an earlier spawning period, are, except for size, in most respects indistinguishable from anadromous sockeye at maturity. In other waters future study may well discover intergradation between kokanee and residual types, while even at Cultus lake the possibility that some anadromy may occur among the young of the kokanee has not been definitely disproved.

THE RESIDUAL SOCKEYE

Samples taken of Residual Populations

The samples which have been studied of the residual sockeye of Cultus lake were for the most part taken incidentally to other investigations on the lake. In 1932 setting of gill nets was begun on the lake, and continued on a limited experimental scale up to 1934. From June, 1935, to the end of 1937 a much more intense fishery was maintained, which had as its primary object a reduction in the abundance of certain fish of the lake. The catch of residual sockeye in the various months and years is as shown in table I.

TABLE I. Catch of residual sockeye in various months and years. A dash (–) indicates that no netting was done during the month in question. Relatively few sets were made in any month of 1932, 1933 or 1934 or in months marked ($^+$) thereafter; $\#$ indicates a half-month only.

	1	2	3	4	5	6	7	8	9	10	11	12	Total
1932	0	0	0	0	0	0	0	0	0	1	47	0	48
1933	0	0	0	0	0	0	0	0	–	1	–	–	1
1934	–	–	–	–	–	3	7	3	8	38	–	–	59
1935	–	–	–	–	0$^+$	1	12	4	16	39$\#$	–	–	72
1936	–	–	3	4	3	3	6	23	48	494	331	37	952
1937	0	0	0	0	2	2	0$\#$	0$\#$	1	14	1$^+$	0$^+$	20

It will be noticed that there are considerable intervals during which no netting was done—a condition determined sometimes by lack of facilities, sometimes by the presence of adult anadromous salmon in the lake. The latter would readily become entangled in the webbing, thereby shortening their own lives and that of the nets.

Gill nets being a selective type of fishing gear, the size of the residual sockeye obtained in any year will depend to a great extent upon the size of the nets in use. The sizes of mesh employed throughout the five years, with the average length and (in brackets) the number of residual sockeye taken by each, are shown in table II. Mesh sizes are given in "stretched" measure, i.e., the distance between every second knot. For the 1936 season a division of the catch was made according to length of fish, (a) including individuals up to 247 mm. long

and (b), fish 248 mm. long and over.   Nets of 102 and 114 mm. mesh were also used but caught very few sockeye.

TABLE II.   Sizes of mesh employed 1932 to 1937 inclusive, with average length and number of residual sockeye taken in each.

Mesh inches	1½	1¾	2	2¼	2½	2¾	3	3½
millimetres	38	44	51	57	64	70	76	89
1932	174(7)	189(37)	199(1)	(0)	(0)	(0)	352(1)	(0)
1934	(0)		220(8)	231(9)	236(7)	246(8)	264(2)	378(2)
1935			315(1)	278(3)	302(1)	293(18)	294(15)	280(1)
1936(a)			214(56)	229(18)	231(31)	238(7)	(0)	(0)
1936(b)			281(38)	286(37)	287(133)	294(431)	295(30)	300(14)
1937			204(2)	312(4)	298(3)	287(5)	335(1)	

The relative amounts of the different meshes in use were exactly the same in 1932 and 1934, and approximately the same in later years (except the 57 mm. mesh, of which only about one-third as much was used in 1936 as of the others). This fact simplifies to some extent the comparison of sizes attained by residuals in different years.   On the other hand, the lower size ranges of residuals were inadequately sampled, except possibly in 1932, so that the length frequency distribution obtained by lumping the catches of all meshes in a given year may differ considerably from the true lengths of that year's population.   In particular, the smaller members of the size groups present in 1934 and 1936 are undoubtedly too thinly represented in the sample, while in 1935 a complete size group might be entirely unrepresented.

SEX RATIO

The residual sockeye captured in every year have been predominantly male.   Among maturing specimens taken prior to 1935 there were no females at all, but in 1935 two females were found among 72 specimens.   In 1936 there were 63 females among 952 fish caught, a fraction of 0.066, or one in fifteen (see table III for analysis as to month of capture and sexual condition).   In 1937 a single immature female was found among 20 individuals taken.

TABLE III. Sex of residual sockeye captured in 1936.

	Maturing this season		Not maturing this season		Sexual condition not recorded	
	♂	♀	♂	♀	♂	♀
March	–	–	–	–	2	1
April	–	–	–	–	4	–
May	–	–	–	–	3	–
June	3	–	–	–	–	–
July	5	–	1	–	–	–
August	15	4	3	1	–	–
September	42	3	3	–	–	–
October	462	29	3	–	–	–
November	308	23	–	–	–	–
December	31	–	4	2	–	–
Total	866	59	14	3	9	1

Several factors may be operative in producing this apparent disproportion of sexes. Net selectivity may be deemed a possible cause, as the male residual sockeye, when mature, have a deeper body, more hooked snout and larger teeth than have females. Hence they might become entangled more readily. However, in 1936 the sex ratio among maturing residuals, taken before the secondary sexual characters are pronounced (June to September), is not *significantly* greater than the ratio among mature spawners (October to December)—being 0.11 as compared with 0.062. In addition, it is shown later that in the case of the very similar kokanee, the two sexes were caught by these nets in equal numbers. It can be concluded therefore that the unequal sex ratio is a real characteristic of the lake's population which net selectivity accentuates only slightly, if at all.

It is shown later that male residual sockeye mature at an earlier age, on the average, than do the females. The latter are therefore exposed to the hazards of lacustrine life for a longer time, and this fact makes for fewer females among the maturing fish. However, even when the numbers of males and of females *of the same age* are compared, it is found that at every age the males greatly exceed the females.

In all probability, therefore, the explanation of the predominant maleness of residual sockeye is to be found in a segregation of the sexes at the time the anadromous stock goes down to sea. A discussion of the facts bearing upon this view is given below, following the presentation of data concerning the size and age composition of residual stocks.

In being predominantly male, the mature residual sockeye differ from the mature anadromous sockeye of the same stock, which at Cultus lake have, usually, a surplus of females (Foerster 1936b, 1938a). The ratio among sea fish is quite variable, but has never been as abnormal as among the residuals, the greatest observed proportion of females having been 0.75. Since the sex ratio among the seaward migrants ("smolts") is never greatly different from 1:1 (Foerster, unpublished MS.) it is evident that the abnormal ratios among residual and anadromous sockeye are *not* directly complementary—the result of a single act of segregation.

Age at Maturity

Age Composition of Samples Taken, and its Seasonal Change

Scale readings have been made to determine the age of a sample of the residual sockeye taken in each year. The scales of residual sockeye, even those which are sexually mature, are usually complete and lend themselves fairly readily to age determination. Little doubt was felt concerning the age of any of those examined from the catch of 1932 to 1935, or of 1937; but in 1936 there occurred a number, comprising ten per cent of those examined, in which the validity of an annulus indicating a very small first-year growth was in question. In practically all such cases the most probable interpretation was accepted as correct, as this procedure involves less error than would their rejection.

Of 48 mature male residual sockeye taken in 1932, a random sample of 21 was found to consist of fish in their second year (II's). A single specimen,

distinct by reason of its large size, was read as in its fourth year (IV). The single residual male taken in 1933 was in its third year (III). Most of the maturing residuals of September and October, 1934, were examined; 39 were III's, 2 were large specimens in their fourth year. A random sample of 34 males taken in late September and early October, 1935, contained 4 III's and 30 IV's. Two females of the same year were both IV's.

In 1936 residual sockeye were taken throughout the breeding season, but unfortunately they were not continuously sampled for age composition, there being no determinations for November, except of females. Second-year fish were however fairly distinct from older ones as regards size (figure 1); considering them as the length-groups below 250 mm. they comprised about 117 of the 843 maturing males taken from September onward. The great bulk of second-year fish were caught in November and December, and scales were read of 25 taken in the latter month. Of maturing males of the 250 mm. group and longer, a random sample of 53 taken in September, October and December, contained 47 III's and 6 IV's. Twenty-eight maturing females, taken from September to November, contained 12 III's and 16 IV's. Applying these proportions to the total catch, the age composition of the 1936 maturing residuals, taken September to December, is approximately as follows:

	Males		Females	
	Per cent	Number	Per cent	Number
Second year....	14	117	0	0
Third year.....	76	645	43	25
Fourth year....	10	82	57	34

The ages of a few non-maturing lake sockeye were also determined, in 1936. Seven males included 6 III's and 1 II—the last still a potential smolt. Three females were all III's.

Of 15 maturing male specimens, taken in late September and October, 1937, 3 were read as II's, 4 as III's, and 8 as IV's; while an additional November specimen was probably IV.

VARIATIONS IN AGE COMPOSITION FROM YEAR TO YEAR

In the 1936 catch it was shown that the proportion of II's in a sample varied with the time it was taken,—this age having been scarce at the beginning, and dominant at the end of the spawning season. Hence the samples of other years, which do not include the whole spawning season, are not likely to represent accurately the total population of those years. But making every allowance for this limitation, it is evident that the age-composition of the residual population varies a great deal from year to year. As compared with 1936, 1932 had relatively more II's and fewer III's; 1935 and 1937 contained relatively a great many more IV's, and fewer III's; while no difference in age composition can be demonstrated between 1936 and 1934.

Part of the variation in the proportion of the different age classes in different years can almost certainly be ascribed to variations in the number of eggs or

fry liberated into the lake, but this does not account for the whole of the observed variability. In table IV is presented a résumé of fish-cultural operations in recent years, taken from Foerster (1936b and MS.), together with an estimate of the absolute abundance of the different classes of smolts and of residuals resulting therefrom. In spite of the incompleteness of the record, it appears probable that the correlation between number of yearling smolts produced and number of residuals resulting from any given spawning is not particularly good. The most glaring contrast involves the 1929 operations, which produced fewer third-year residuals than other years having smaller smolt migrations. Nor is there any consistent relationship between the number of two-year old smolts and number of residuals.

TABLE IV. Smolt and residual sockeye resulting from the spawnings of 1928 to 1935, as far as they have been determined. Roman numerals under "residuals" indicate year of life at maturity. (Data on propagation and smolts from Foerster 1936b and MS.)

Year of spawning	Type propagation	Eggs in females (thousands)	Eggs or fry planted (thousands)	Smolts yearling	2-year	Residuals II	III	IV
1928	Artificial	-	2,650	39	5.2	?	?	few?[+]
1929	"	-	9,093	350	0.2	?	few-none	?
1930	Natural	24,900	-	788	15.9	many	f.c.-few	f.c.?[+]
1931	Both	54,310	6,031	1,529	63.3	?	many	many
1932	Artificial	-	4,825	122	14.2	few?	few	f.c.
1933	"	-	4,372	242	2.2	?	many	f.c.
1934	"	-	5,590	501	1,1	f.c.	few	
1935	Natural	40,000	-	3,094		f.c.?		

f.c. - fairly common.   [+]large specimens.

COMPARISON OF AGES OF RESIDUAL AND ANADROMOUS SOCKEYE

In general it may be said that male residual sockeye mature in fair numbers in their second year of life, in large numbers in their third year, and a certain number, usually small, in their fourth year. Females mature in their third and fourth year. Comparing these with the ages of Cultus lake anadromous sockeye (cf. Foerster 1929a and b) we find that the latter mature principally as III, IV and V year fish. The IV group contains the great majority of fish of both sexes, the proportion of III's being greater, and V's less, among males than among females. In the case of both sexes, therefore, the residual stock tends to mature at an earlier age than does the anadromous.

SIZE AT MATURITY

All residual sockeye taken in the nets have been measured while still fresh. Length was taken from tip of snout to fork of tail. In table V is presented a résumé of the measurements of fish of known age, taken in different years, while in figure 1 are plotted the length frequency distributions of the total catch in

each year, except 1933, up to 1936. In 1933 the single residual measured 255 mm. Sizes of 1937 autumn residuals were as follows: 200 mm. group—1, 205—2, 260—1, 265—1, 270—1, 290—1, 295—3, 315—2, 320—1, 325—2, 330—1, 335—1.

TABLE V. Average length, range of length, and standard deviation in length, in millimetres, of mature or maturing residual sockeye, taken from September to December in various years.

Year of maturity	Number examined	Year of life	Sex	Mean length	Range	Standard deviation
1932	46	II	♂	187	162 – 222	13.1
	1	IV	♂	352	–	–
1933	1	III	♂	255	–	–
1934	41	III	♂	232	200 – 272	16.8
	2	IV	♂	376	370 – 382	–
1935	4	III	♂	250	225 – 270	19.3
	30	IV	♂	293	265 – 310	11.8
	1	IV	♀	266	–	–
1936	25	II	♂	213	180 – 240	15.3
	48	III	♂	289	200*– 330	15.6
	6	IV	♂	302	270 – 325	21.2
	12	III	♀	288	265 – 330	19.2
	16	IV	♀	295	275 – 320	11.8
1937	3	II	♂	222	202 – 260	30.3
	4	III	♂	281	266 – 297	13.1
	9	IV	♂	318	296 – 335	13.8

*This fish seemed very clearly a III. The measurement was however not included in calculating the mean and standard deviation, as it differs from the former by six times the latter. The next smallest fish in this group measured 265 mm.

The limitations of the data from which these summaries have been made have already been discussed, but with due caution they can be used for a discussion of the following points:

CHANGE IN SIZE WITH PROGRESSION OF THE SEASON

In 1936 the lake was netted throughout the whole period of maturity of the residuals, and with a similar assortment of nets during the entire season. In figure 2 are shown the length-frequency histograms of the total catch, divided into semi-monthly periods beginning October 1. It is evident that the average size of mature residuals decreases rapidly from the beginning of November onward. At the same time the histograms become definitely bimodal, with a trough about 250 mm. There is good reason to believe therefore that the decrease in average length is principally because second-year fish first appear in good numbers at a time when the III's are beginning to decrease in abundance. The change in age-composition of the population continues throughout November, so that by December 1 very few III's remain. Considering fish longer

than 247 mm. as older than II's, it appears likely that the average size of such decreases only slightly, if at all, throughout the season. The II's, on the other hand, from the time of their first appearance, decrease continuously in average length.

OVERLAPPING BETWEEN AGE-GROUPS

Male residuals maturing in the same year, but of different ages, may or may not overlap in length. Second-year residuals in 1936 were fairly well
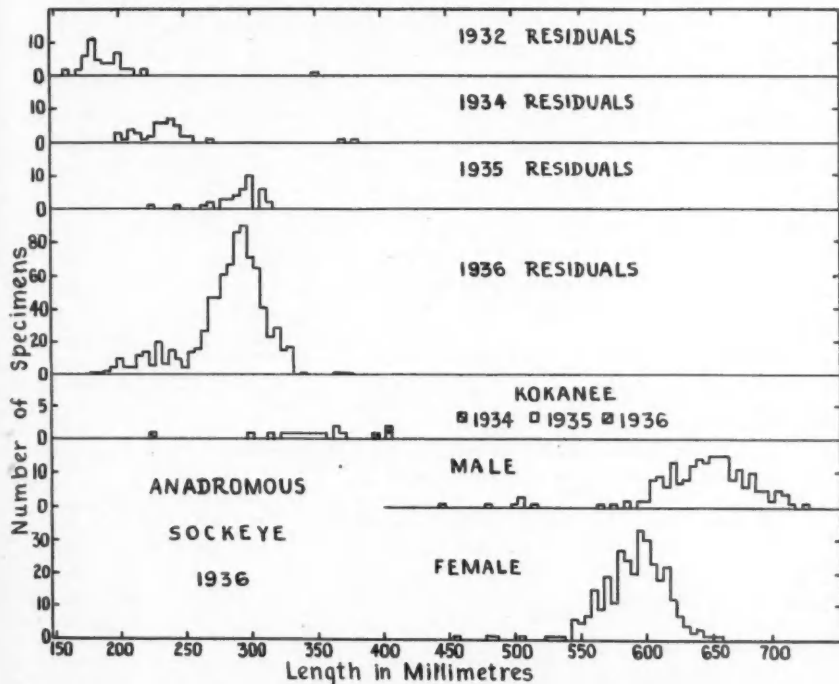

FIGURE 1. Length frequency distribution of the residual sockeye of 1932, 1934, 1935 and 1936. of the kokanee of 1934, 1935 and 1936, and of the male and female anadromous sockeye of 1936. For the lengths of residuals in 1937, see the text.

separated from the III's, but not completely so. In the same year, the III's and IV's of both sexes overlapped over a large part of their length ranges, although the older fish averaged slightly larger in each case. In 1935 and 1937 IV's were apparently better separated from the younger group, and the exceptionally large IV's of 1932 and 1934 were very distinct from any other age.

SIZE IN DIFFERENT YEARS

Fish of the same age, but taken in different years, appear to be quite variable as to size. The II's taken in 1932 averaged 26 mm. shorter than those whose

age was determined in 1936. Although the use of smaller nets in 1936 would undoubtedly have reduced the average size, this is to some extent compensated by the fact that the 1932 catch was made on November 18, hence by analogy with 1936 probably consisted of the larger earlier II's of that year. The III's of 1934 were the smallest observed, being 56 mm. less than those of 1936, with 1935 probably intermediate. The IV's increase in average size in this order: 1935, 1936, 1937. In addition, there were in 1932 and 1934 a few IV residuals of exceptional size, whose origin is discussed later.
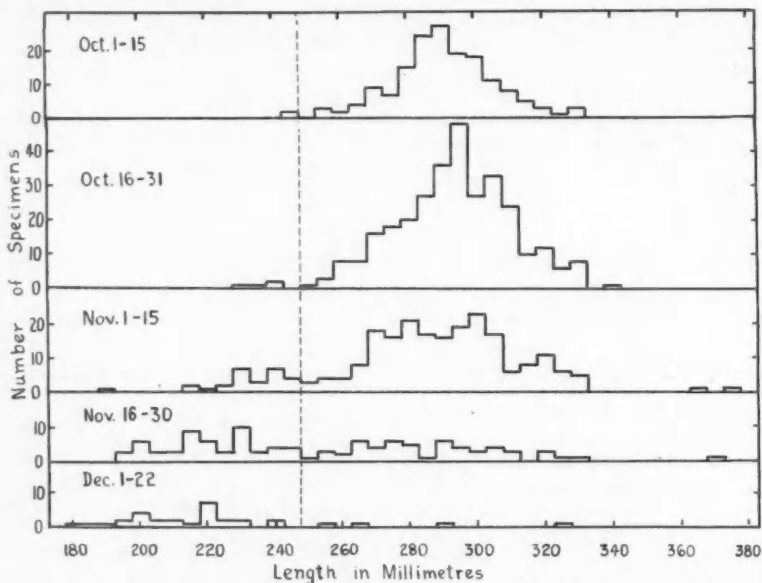
FIGURE 2. Length frequency distributions of the residual sockeye taken in 1936, arranged in semi-monthly periods beginning October 1. The vertical dotted line indicates the approximate length which divides second-year from older fish.

COMPARISON OF FISH OF THE SAME YEAR-CLASS IN SUCCESSIVE SEASONS

This can be done, with any approach to accuracy, in only two cases. The IV's of 1935 averaged 60 mm. longer than the III's of 1934; the IV's of 1937 29 mm. longer than the III's of 1936, considering males only in both cases.

COMPARISON OF RESIDUALS AND SMOLTS OF THE SAME YEAR-CLASS

In figure 3A are plotted the average lengths of III residuals against that of year-old smolts of the same spawning; figure 3B shows the same for IV residuals, excluding the few very large ones of 1932 and 1934; figure 3C shows the relation between length of year-old smolts and the average length attained by III residuals at the completion of winter growth at one year of age (found from scale reading, table VI). The points as plotted suggest that, in general, spawnings

which produce small smolts tend to produce small residuals, and vice versa. The coefficients of correlation are calculated in each case, and indicated on the graphs. From Fisher (1932, table VA) it is known that, for four pairs of observations, a coefficient must be at least 0.90 to have 9 chances in 10 of being significant; for three pairs it must be 0.988. One of these attains this level of probability, so that the apparent relationship can on this evidence be considered suggestive. Data for more years would be necessary to finally determine whether it is real or accidental.
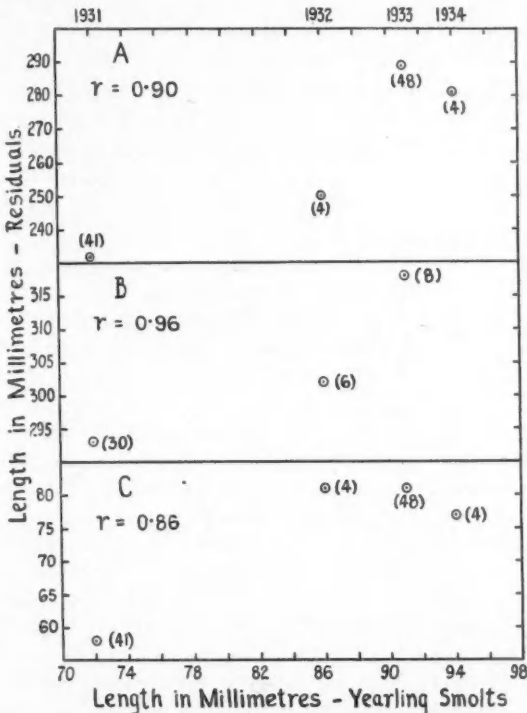

FIGURE 3. Relation between average length of yearling smolts and the average length of residual sockeye of the same spawning. A. Total length of III residuals. B. Total length of IV residuals. C. Calculated length of III residuals at the end of their first year of growth. Correlation coefficients (r) are given for each group. Bracketed figures indicate the number of specimens upon which the determination is based. The years indicated at the top are the time of spawning of the year-class represented by points lying below them.

COMPARISON OF LENGTHS OF RESIDUAL AND ANADROMOUS SOCKEYE

In figure 1 is presented a comparison of the lengths of the residuals of different years with the mature anadromous sockeye of 1936. The latter consisted principally of IV-year fish, a few smaller III's, and a few V's—the last not easily separable from IV's by length. It is evident that the residual sockeye

are much smaller than the anadromous. The largest residual taken was 382 mm. long; the smallest anadromous sockeye of 1936 was 445 mm.; and the former was a year older than the latter.

WEIGHTS OF RESIDUALS

The residual sockeye taken from 1932 to 1934 were weighed while still fresh. Weights of mature specimens varied from 38 grams (0.08 lb.) for a 1932 fish 162 mm. long, to 636 grams (1.4 lb.) for the 1934 specimen 382 mm. long.

PARENTAGE

Residual sockeye have been defined as the non-migratory progeny of anadromous parents. It is now necessary to present the evidence that the Cultus lake fish under discussion are really residuals as defined.

A very suggestive fact is the scarcity of females among them. If the few females present in any year are considered as the mothers of the II, III and IV-year residuals taken in subsequent years, then there must be in effect somewhere in their life-history a mechanism capable of producing a preponderance of males which is without parallel among salmonoid fishes. The existence of such a mechanism, whether genetic or ecological, appears most improbable.

It has been found by Foerster (1938b) that, of artificially-reared sockeye released in the lake immediately before migration time, not all would go down to sea. Only 63, 66 and 33 per cent respectively of those so released in 1931, 1933 and 1935 became smolts. The remainder must either have perished prematurely in the lake, or have stayed there to become residuals. Since these fish were in every case much larger than wild sockeye of the same brood, they would probably mature mostly during the same year as liberated, if the analysis given under "Rate of growth" below is valid. Unfortunately no netting was done in the lake in late autumn of these years, so the principal chance for their recovery was lost. However, in October, 1934, a III residual was taken, which was also one of the fish marked and liberated on April 1, 1933. It was noticed as an exceptionally large but otherwise normal individual among a school of about 150 unmarked III residuals taken at Smith Falls hatchery. Thus the fact that the progeny of anadromous sockeye can mature in the lake is definitely established.

Female residuals do produce eggs of normal appearance. Their fertility has not been tested, but if they and the milt are viable, there is no reason to suppose they could not produce fingerlings to swell either the natural smolt migration or the residual-type stock. The evidence cited above shows merely that residuals do not propagate their kind *to any significant degree*.

RATE OF GROWTH

Measurements were made of the width of the annuli on the scales of sockeye whose age was determined, and from them the length at the time of completion of each winter ring was calculated. As in the work of Foerster (1929c), measurements were made from the first circulus laid down, rather than from the centre

of the scale, and the size at that time was considered to be 38 mm. throughout. After subtracting this quantity from the total length of the fish, a linear relationship was assumed between growth of fish and growth of scale.

Average annual increase in length of the various kinds of specimens is shown in table VI, and in figure 4 the cumulative growths of different groups are compared. From graphs A and B it is apparent that in 1936 specimens of either sex, which were maturing at a later age, have a slower average rate of growth than those which matured earlier. In graphs C and D are compared the growths of male fish of the same age at maturity, but taken in different years. In graph E is compared the rate of growth of III- and IV-year maturing males of the same year-class, showing that the first-year growth of the two types was the same, but that the older fish grew more slowly subsequently. The same is more strikingly shown in graph F, which compares maturing and non-maturing third-year fish of the same year-class, taken in 1936.

TABLE VI. Average increase in length in millimetres, of residual sockeye, from the time the first circulus appears on the scale. The growth in the final year of the fish's life terminates with capture in autumn, i.e. does not include a whole growth-year. The standard deviation (σ) is of the individuals, not of the mean.

Year of capture	Sexual condition	Sex	Age	No. examined	1st year growth		2nd year growth		3rd year growth		4th year growth		Length when caught	
					mean	σ	mean	σ	mean	σ	mean	σ	mean	σ
1932	Maturing	♂	II	21	65	9.4	79	12.2					182	12.5
1934	Maturing	♂	III	41	20	4.0	73	10.1	101	16.3			232	16.8
	Not maturing	♂	III	1	18	–	55	–	121	–			233	–
1935	Maturing	♂	III	4	43	6.3	90	22.1	78	14.5			250	19.3
	"	♂	IV	30	20	5.8	61	6.7	102	11.3	73	11.1	293	11.8
1936	Maturing	♂	II	25	64	9.0	111	16.3					213	15.3
	"	♂	III	48	43	13.6	96	15.9	112	20.6			281	15.6
	"	♂	IV	6	84	8.5	64	8.2	86	18.4	90	23.2	302	21.2
1936	Maturing	♀	III	12	36	10.7	105	21.8	109	18.0			288	19.2
	"	♀	IV	16	23	5.6	61	9.8	82	15.6	91	13.8	295	11.8
1936	Not maturing	♂	II	1	48		84						170	
	"	♂	III	6	40	5.8	70	14.5	104	19.8			253	30.7
1936	Not maturing	♀	III	3	33	12.3	91	11.4	127	13.4			289	19.0
1937	Maturing	♂	II	3	59	20.1	125	21.3					222	30.3
	"	♂	III	4	39	4.1	120	14.4	84	20.5			281	13.1
	"	♂	IV	8*	43	7.5	79	19.2	103	18.2	55	–	318	13.8

Seven of these had part of the fourth year growth eroded. Calculation of increments for the others was made by assuming a constant ratio of fourth year to other growth.

Foerster's studies (1929c, 1934, 1936a) of the growth of migrating smolts from Cultus lake has shown that those which migrate at two years of age have had, during their first year, a smaller average growth than those of the same year-class which migrate as yearlings. A comparison of smolts and residual sockeye of the same year-classes is made in table VII, in respect to their average rate of growth in the same seasons. It will be observed that the residuals maturing in their second year exhibit the greatest growth, during their first year, of any type of fish. Those of the spawning of 1930 averaged 13 mm. longer than the yearling smolts, as judged from a sample taken November 18; those

of the 1934 spawning averaged 7 mm. longer, the sample having been taken from the small residuals of December, hence probably giving too low a value to be representative.   On the other hand, residuals maturing in their third or fourth year made *less* growth during their first year than did yearling smolts: 14 mm. less in the case of the 1931 spawning, and 11 mm. less in the case of the
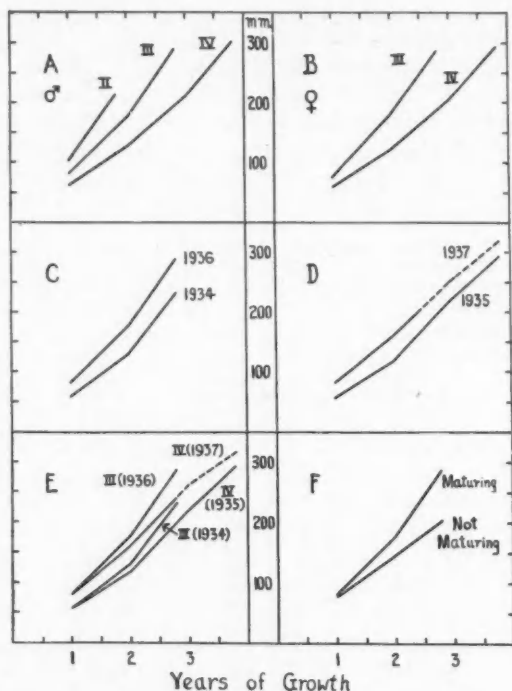

FIGURE 4.   Calculated average growth, in millimetres, of residual sockeye.   A and B. Males and females respectively of the different year-classes of mature residuals taken in 1936. C. Third-year residuals.   D. Fourth-year residuals.   E. Comparison of residuals of the same year-class but of different age.   F. Maturing and non-maturing residuals of the same year-class, taken in the fall of 1936.   Roman numerals indicate year of life at maturity.   (Arabic numerals on the abcissal scale indicate the time of completion of a season's winter growth annulus, in April.   Growth from that time to the autumn, in the final year, is plotted as equivalent to four-fifths of a year's total growth).

III's of the 1933 spawning, to cite the best authenticated cases.   (It will be remembered that the III residuals of both these years were somewhat inadequately sampled in the lower part of their size range, hence the real difference is slightly greater than the above).

Finally must be mentioned the residuals of exceptionally large size.   These were all males, and consist of a IV in 1932 (352 mm.); two IV's in 1934 (370 and 382); a III taken in July, 1936 (342); and three of unknown age taken in

November of the same year (365, 370, 375), which were separated from known III's and IV's of that autumn by a considerable length interval (figure 2). Of these, the 1932 and 1934 fish had the scale edges considerably eroded, so length increments could not be accurately calculated, but it was noticed that the first-year growth had been great—relatively much greater than in the case of other IV's. The length of the III of 1936 was calculated as follows: 1st year—97 mm., 2nd—221. Comparing with the other fish of the 1933 spawning (table VII), it appears that its first-year growth exceeded that of all other groups listed, and would be of the same order as the II residuals, if any such were present.

TABLE VII. Length attained at end of first and second growth-years, by smolts and residuals of various year-classes. Data are for males only, except in case of all year-old smolts, and the two-year-old smolts of 1933 and 1934 spawnings. Data for smolts are from Foerster (unpublished MS.).

| Year of spawning | Type of fish | Age 1 year | | Age 2 years | | No. of specimens |
		length	year's increment	length	year's increment	
1930	Year-old smolt	90	52			many
	2-year-old smolt	63	25	122	59	10
	Residual II	103	65			21
1931	Year-old smolt	72	34			many
	2-year-old smolt	51	13	104	53	49
	Residual III	58	20	131	73	41
	Residual IV	58	20	119	61	30
1932	Year-old smolt .	86	48			many
	2-year-old smolt	65	27	124	59	37
	Residual III	81	43	171	90	4
	Residual IV	62	24	126	64	6
1933	Year-old smolt	91	53			many
	2-year-old smolt	70	32	119	50	35
	Residual III	80	42	177	97	48
	Residual IV	81	43	160	79	8[*]
1934	Year-old smolt	95	57			many
	2-year-old smolt	74	36	124	50	6
	Residual II	102+	64+			25
	Residual III	77	39	197	120	4

[*]See note to table III.

SEGREGATION OF RESIDUALS AND SMOLTS

The kinds of sockeye produced from each spawning of the anadromous stock are diagrammatically indicated in figure 5. It will be observed that sockeye of a given year-class, approaching migration time in their second year of life, can be divided into three overlapping length groups on the basis of the fish

included.   A group of fish (*a*) of large average size will remain in the lake and mature there; a group (*b*) of intermediate size will migrate as yearling smolts; and a group (*c*) of small average size remains in the lake.

Of group *a*, the majority appear to mature the same autumn, as II residuals (group *d*).   A few at least survive to mature the following year (group *i*), and are represented in the data by the large III residual of the 1932 spawning, and the marked specimen mentioned above.   Still others mature only in their fourth year (group *l*), as shown by the large IV residuals of 1928 and 1930 spawnings. It is noteworthy that in 1930 these large specimens were probably not uncommon (the two specimens were taken in a single night's fishing), and occurred in a cycle which also had an abundance of II residuals.

Sockeye of group *c* are further segregated at migration time during their second year of age.   A group of large fish (*e* + *f*) remains in the lake, and these
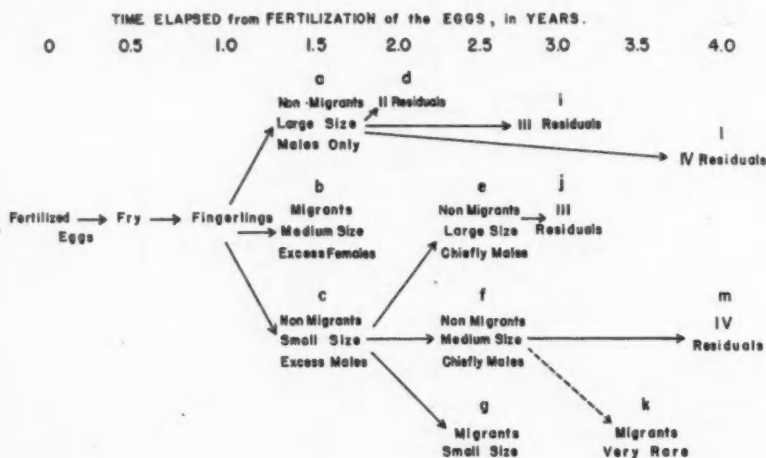

FIGURE 5.   Kinds of migrant and residual sockeye which may be produced from the spawning of the anadromous stock in Cultus lake.

are already somewhat divided as to size: the larger of them tend to mature the same year, as III residuals (*j*), while the smaller stay through another year and mature as IV residuals (*m*).   The smallest fish resulting from group *c* are migratory, the two-year-old smolts (*g*).

Three-year-old smolts (*k*) are included in figure 5 on the basis of a single male specimen, from the spawning of 1932, taken in the seaward migration of 1936.   Its calculated size was: 1st year—64 mm., 2nd—141, 3rd (time of capture) —216.   The first year's growth is close to the average of group *c* of that cycle (table VII), while the second seems closest to group *f*, as far as can be learned from the few representatives examined.   In default of better information therefore it has been indicated as closest to the IV residuals of type *m*.   In more northern lakes however 3-year-old smolts are one of the more common types,

so from analogy with the first segregation, it is possible they spring from a group of smaller average size than group g in the second segregation, which group might also give off an additional class of IV residuals.

The sex of the various groups may be summarized as follows: Derivatives of group a have been exclusively male. Yearling smolts (b) are of both sexes, and Foerster (unpublished MS.) who examined their sex ratio in several years, found little variation from 1:1, although those from the spawning of 1933 showed a significant excess of females (0.532) among 1500 examined. Residual III's of group j are predominantly male, the highest observed fraction of females being 0.066, among fish of the 1933 spawning. Residual IV's of group m are also predominantly male, although among them is found the greatest fraction of females as yet observed: about 0.3 of those of the 1932 spawning. Two-year-old smolts (g) have also been studied by Foerster, again without discovering any general tendency away from a normal sex-ratio. In years when their numbers were small (cf. table V) not enough were examined to provide any reliable guide, but of the spawnings of 1927, 1931 and 1932, there were 288, 505 and 116 respectively available, and only among the first-named was there a significant departure from the normal ratio—a proportion of females of 0.59.

SIZE, SEX AND MIGRATORY IMPULSE

The rather complex segregation, by size and sex, which is indicated above, has provided material for the development of a tentative explanatory hypothesis, which assumes the following: (1) that the forces which bring about migration operate more strongly as the young sockeye increase in size, and for fish of a given size somewhat more strongly on females than on males; and (2) that after a certain size is reached, something connected with approaching maturity inhibits the effect of (1), this latter influence being much stronger among males than among females. The relative and absolute influences of factors (1) and (2) are not identical among all fish of the same size and sex, but vary somewhat, so that the size classes they segregate overlap to a considerable extent.

The influence of factor (1) alone would divide any population of yearlings approaching migration time into a group of large migrants containing an excess of females, and a group of small non-migrants containing an excess of males (group c). The added influence of (2) would further divide the former group into large non-migrants, predominantly or exclusively male (group a), and migrants of intermediate size and of both sexes, with a preponderance of females (group b). If the a and c groups are together small in number as compared with b, their defection will produce no detectable change in the sex ratio of the latter, in samples of reasonable size. The same will be true if c is fairly large, but contains no very great excess of males.

In the following year, group c again comes under the influence of these factors. Its members have greatly increased in size, so that none are so small as not to be affected by factor (1), and when it predominates the fish become two-year-old smolts. Among the larger fish however its effect is suppressed by factor (2), so that they remain in the lake and become residuals. This will

apply especially to the males, but there are a few females too which do not migrate. On this theory, then, the sex ratio of two-year-smolts could be quite varied, as it is the end product of two independent segregations, the first preferring males, the second females. The fact that it is not far from 1:1 among the larger populations of those smolts, suggests that in these cases the ratio of III and IV residuals to two-year-old smolts is not very great, and also that group c in those years did not contain any very great excess of males.

With the above hypothesis it appears possible to arrange in some kind of order an otherwise chaotic mass of data, and to that extent it deserves consideration. There is the following additional evidence in its favour.

(1) The assumption that factors influencing migration act more strongly on larger fish derives support from the fact, shown by Foerster (unpublished MS.), that in most yearling migrations, larger fish, of both sexes, appear earlier on the average than do smaller ones. The same applies to two-year-old migrants.

(2) That such factors act more strongly on female fish is suggested by the observation during the migration of 1937, that females were in significant excess during the first half of the run, and males in the last half, although the average size of the whole sample of each sex was the same, and the ratio of their numbers did not significantly differ from 1:1.

(3) That size, when increased beyond a certain limit, tends to reduce percentage of migration has been demonstrated by Foerster (1938b) in the case of experimental fish. Different groups of pond-raised sockeye marked and released at intervals during the season 1934-35 showed increasing survival to the smolt stage, up to and excepting the last and largest group released. This group yielded only 60 per cent of the smolt production of the previous one, and 50 per cent of what was expected of it from extrapolating earlier returns. Further, the smolts produced averaged 4 mm. *less* in length than the sample measured at the time of their release into the lake, although they had been at large for $1\frac{1}{2}$ months and *individually* must have increased in length by an average of 5 mm. at least, to judge by the growth of the group marked earlier. It is probable therefore that the difference in average length of the migrants and the non-migrants of this marking was at least 18 mm.

(4) That this upper size limit to migration is more effective in the case of males than of females is rather weakly suggested by this same group of marked migrants, of which a sample of 128 yielded a proportion of males of 0.438. Owing to two different sampling ratios having been used, the standard deviation of this result cannot be exactly estimated, but it is such that the difference from 0.5 shows a probability of significance of about 0.8. This may be said to be somewhat favourable to the hypothesis; at the least, it is not unfavourable. In comparison, fish of the previous marking had a proportion of males very close to 0.5 at time of migration: 0.505 of 140 fish examined.

(5) The hypothesis demands that there should be among year-old migrant smolts an excess of females, though it be only a small one and likely variable from year to year; provided of course that in the whole population just prior

to migration there are equal numbers of the two sexes. It is therefore interesting to observe in one year a significant excess of females among such smolts. This in itself does not establish the hypothesis, but the opposite observation—an excess of males—would have made it untenable.

(6) Consideration of the numbers of the different types of smolts and residuals produced by successive spawnings (table IV), and their size (tables VI and VII) seems to show no discrepancies between data and hypothesis, and in some respects the data may be said to support it. The three groups of the first segregation (a, b and c), and of the second (e, f and g) are listed below for each spawning, abundant and fairly common groups being in italics, rare or absent groups in ordinary type, while those for which no information is available are queried:

1930 (*a*, *b*, *c*; e, f, g)   1931 (a? *b*, *c*; e, *f*, g)
1932 (a, *b*, *c*; e, *f*, g)   1933 (a? *b*, *c*; e, *f*, g)
1934 (*a*, *b*, c?; e, f?, g)

Considering each segregation separately, we could expect an abundance of either all three possible types, or of two adjacent ones, or of only a single one; but the abundant occurrence of two extreme size groups along with scarcity of the intermediate one would be tantamount to disproving the existence of the type of segregation in question. Actually no such case occurs.

The year 1930 may seem an exception, as there were abundant II residuals (*d*) and two-year-old migrants (*g*), representing the two extremes of size of the *two* segregations. It must be remembered, however, that the two segregations are independent of each other, being based on size of the fish at the age of 1 and 2 years respectively. The class *c* fish of the 1930 spawning suffered, during their second year, from the competition of an exceptionally numerous population of fingerlings, which appears to have seriously reduced the food available (Ricker 1937) and slowed up their growth. This is reflected in the fact that the percentage increase in length, during their second growth-year, of two-year-old smolts resulting therefrom, was no greater than that of two-year-old smolts of other years (table VI), although the latter represent only the smaller fish of their original *c* groups, the larger ones having become III and IV residuals.

Colour at Maturity

In residual sockeye of the male sex, the colour of the back and sides changes at maturity to olive-grey with fine black spots, and the whites below become very dusky. Females are similar, but depart less from the bright blue-grey and silver immature colours. Although the flesh of both sexes is the usual rich salmon tint, we have not observed any trace of red colour externally. All this is in marked contrast to the maturing anadromous salmon of the same brood. In these the male has a light green head, sharply marked off from the trunk, which is crimson on back and sides, becoming scarlet posteriorly. Females also are often red, but duller, or they may have tints of purple, green or yellow in irregular patterns. That the lack of colour in the residuals is an effect, directly or indirectly, of their lacustrine existence, must be granted. Curiously

however, the kokanee, which also spend their whole life in the lake, are even more brightly coloured than the sea-run sockeye.

Other characteristics of the mature male sockeye—thin body, humped back, elongate and curved maxillae, and enlarged teeth—are well developed in the residual stock, although perhaps somewhat less relatively than in the case of the anadromous or kokanee populations.

Susceptibility to Salmincola

The copepod parasite *Salmincola gibber* infests the skin of lake sockeye about the gills and fins. Foerster (1929c) has described their occurrence upon migrating smolts in 1926 and 1927. In various migrations since that time the infestation has varied from heavy to almost none. Residual sockeye, almost without exception, have carried a medium to heavy infestation of *Salmincola*. Their attack was apparently heaviest in 1936, when very few residuals were taken which had less than 20 of the parasites, and 40 was not at all an uncommon number. Damage to gills was plainly evident in a shortening of the filaments and thickening of their tips, and many times the fins too were more or less frayed. Having in mind the possibility of excess mortality among females, all such were carefully examined, but they seemed to suffer no more than the males. The surprising fact is that, in spite of their heavy load of parasites, all residual sockeye taken before the spawning season were fat and apparently in good condition.

Time of Spawning

In figure 6 are plotted the weekly catches of residual sockeye for 1936 as well as the number of male and female anadromous sockeye killed or dead during the spawning operations at the lake's outlet. The residual sockeye apparently first tend to move inshore and toward the bottom about the end of August, but this migration reaches no significant proportions and cannot be confirmed before the last two weeks of September. It is at the latter time also that the morphological changes of the maturing males first become apparent. The first ripe male, exuding milt, was taken October 6; a week later about half of those taken were ripe, and by the 21st nearly all. Females matured at approximately the same time.

From figure 6 it appears that the time of maturity of sea-run sockeye has its mode somewhat later than that of the residuals. *How much* later is difficult to say, for the two sets of data are not altogether comparable. The histogram for residuals, even discounting non-mature fish up to mid-October, tends to be shifted earlier in the year, for it is a record of the capture of *live* fish, whereas that for sea-run sockeye is for the most part a record of fish killed when their spawning was over. Probably the average time of spawning of residual sockeye precedes that of anadromous sockeye by no more than two weeks.

Food

The food of a number of residual sockeye in their second and third year of life has been tabulated in an earlier paper (Ricker 1937). In general they eat

the same organisms as do the smaller sockeye: pelagic plankton Crustacea, and especially *Daphnia*. Aquatic and terrestrial insects are taken to some extent, but are of minor importance. In a few cases small fish were eaten, including small fry of *Cottus asper*, and recently a few fourth-year specimens have been found to consume small sockeye fry.

In order to obtain *Daphnia* in large numbers in summer, it was shown that the feeding of lake sockeye must be principally in the region of the thermocline, i.e. at depths between 9 and 15 metres. The occasional occurrence of large numbers of *Epischura* in the stomachs indicate, however, that foraging expedi-
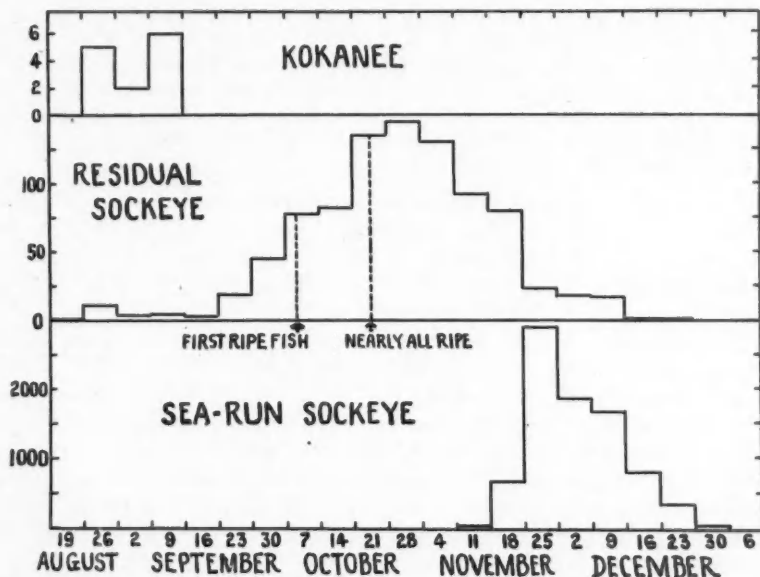
FIGURE 6. Catch by weeks of kokanee in 1935, of residual sockeye in 1936, and of anadromous sockeye in 1936.

tions into the epilimnion (where it is concentrated) are sometimes made, and the terrestrial items are presumably taken from the surface of the water.

HABITS

The residual sockeye which are taken in spring and summer are found in the deeper parts of the lake—from 15 or 20 metres down to the maximum of about 40. As suggested above, their preferred level is probably up off the bottom, so that their capture in a bottom-set net at this time is to be regarded as an exceptional occurrence. With approaching maturity there is associated a tendency to frequent lower levels, to judge by the greatly increased catches of late September and early October, before the true breeding season. At the same time some of them move to shallower water, and later on they may be taken

close inshore around practically the whole of the lake's periphery. A few may even run up creeks tributary to the lake, as about 150 were taken in a small hatchery tail-race in 1934; but the number of such suitable tributaries is limited to one or two. Ripe individuals are however also taken in deep water. In 1932, when natural spawning of anadromous sockeye was permitted in the lake, ripe residual specimens were taken from the same redds as the larger fish. Whether or not they breed with the sea fish is not known.

ECONOMIC POSITION

As a game fish, the residual sockeye are worthy of some regard. They take a spoon fairly readily, and afford Cultus lake anglers some sport in years when they are common, notably in 1936. Although not usually exceeding a pound (½ kg.) in weight, their flavour excels that of any other fish of the lake.

Viewed from the standpoint of the commercial sockeye fisheries, the existence of a residual population may be less desirable. The direct loss lies in the fact that each of the fish maturing in the lake was at one time a potential smolt; its failure to migrate was a potential loss to the fishery. But this is not all. Since the residuals eat the same food as the fingerlings, a large residual population might seriously compete with the latter for the available food, and so reduce their size, vigour and survival rate. In addition, the larger residuals directly destroy some small fingerlings.

In another way, nevertheless, the presence of residual male sockeye may be of inestimable benefit to the lake. White (1935) has evidence that the presence of mature male parr was a factor in attracting Atlantic salmon back to a stream where they were introduced. This, he suggests, may be a reaction to the milt shed into the water. The converse proposition, that salmon will not return to a "parent" stream unless such parr are there, has not yet been established, and neither case has yet been demonstrated for any Pacific salmon. Nevertheless, the possible importance of the residual sockeye in this connection is obvious.

PREVIOUS RECORDS OF RESIDUAL SOCKEYE

Since residual sockeye are by no means rare fish in Cultus lake, it would be most unusual if they were not found in some, perhaps all, other lakes which have an anadromous sockeye population. Perusal of the literature has brought to light two descriptions which can reasonably be interpreted as pertaining to residual fish.

Ward (1932) has described the appearance of a fresh-water type of sockeye in the Baker river system, tributary to the Skagit river in Washington, following construction of a high power dam on it. Although these are called "landlocked sockeye" and described as "corresponding closely to the landlocked variety of that species as found in other waters", Ward postulates, and on good evidence, that they are progeny of the anadromous stock. Hence they are really residual sockeye as we define the term. His description of them shows points of similarity with Cultus lake residuals, viz., a great excess of males; a tendency for this sex

to mature in greatest numbers in its third year of age; and a decidedly duller colour at maturity than have typical kokanee, even though most of the males and some of the females showed some red skin pigment at maturity.

What may be a record of the occurrence of residual sockeye in two lakes of the upper Fraser river under entirely natural conditions is given by Babcock (1903), in the following words:

"The Evermann, or permanently small form of the sockeye salmon, is found in Seton lake in October, and in Anderson lake in November. Their presence there at other times has not been recorded. These small fish annually appear in great numbers in Seton lake, about the middle of October, at that time rising to the surface of the water with the abdomen so distended with gas that they are held there, where they struggle for a few days and then die. Their fins are frayed and covered with fungus, the tail in many specimens being entirely gone. They are of a dark muddy colour, and show dark spots on the back, and never show any of the brilliant red colour which so distinguishes the larger variety. They average about 8 inches in length and weigh only a few ounces. At times the surface of the lake is practically covered with their remains. The Indians term them (oneesh)*, and gather them in great quantities by means of scoop nets when they first come to the surface. Nothing regarding their spawning habits is known. They never enter the creeks, as far as reported. I saw none save in the lake proper. These fish are common to both Seton and Anderson lakes, but come to the surface of the latter some 3 or 4 weeks later than the former."

In reading this account it may be remembered that up to 1902 anadromous sockeye were exceedingly numerous in the Seton-Anderson lake system, (Babcock 1902, 1903), so that the presence of residuals in the large numbers suggested above should not occasion too much surprise. Indeed, the point may be raised whether excessive production of residual, rather than migrant, stock may not have been an intermediate factor setting a limit to the abundance of the runs of sockeye in those days, particularly the largest ones. And in a study of intra-specific predation, competition for food, and other ecological relationships between residual sockeye of one year class, and fingerlings of later years, there may possibly be found a solution of the phenomenon of the "big" cycle years on the Fraser, whose fish in early days so greatly exceeded in abundance those of the other three cycles.

THE KOKANEE

SPECIMENS TAKEN

Kokanee, as here defined, are sockeye which spend their whole life in fresh water, and whose parents, for several generations back, have done the same. They are known to occur in many lakes of British Columbia (Dymond 1936) and adjacent regions. A good description of their breeding behaviour and appearance in lake Washington is given by Schultz (1935).

In Cultus lake kokanee appear to be quite scarce, as compared with the residual sockeye. The experimental netting of 1932 and 1934 did not yield a single specimen. In 1935 rather intensive netting produced 13 specimens, in

*If these fish can be definitely shown to be residuals rather than kokanee, the term *oneesh* will be available as a distinctive indigenous name, to be used in place of the rather awkward descriptive name used in this paper.

1936 only two, in 1937 none.   In addition to these, the male of a spawning pair
was taken by angling on September 18, 1934.

SEX RATIO

Of the 13 kokanees of 1935, only 10 were preserved for examination, and
these consisted of an equal number of the two sexes. In 1936 a single male and
single female were taken.   There is thus no suggestion of the unequal sex ratio
characteristic of residual sockeye.

AGE AT MATURITY

All of the above specimens were mature breeding fish, and had absorbed so
much of the edge of the scales as to make it impossible to obtain good specimens
for age determination.   In this they resemble anadromous and differ from
residual sockeye in similar sexual condition.   Some females yielded scales on
which three areas of rapid summer growth could be identified.   The small
diameter of such scales in relation to the length of the fish makes it seem probable
that a fourth summer had been absorbed entirely.   The evidence available
therefore indicates that the female kokanee commonly mature in their fourth
year of life.   The small male of 1934 had a scale showing only two summer
rings and an absorbed margin.   It was probably in its third year, and from the
size difference, it may be supposed that the males of later years were in their
fourth year.

SIZE AT MATURITY

The data on length are given in table VIII and the length frequency dis-
tribution is plotted in figure 1.   Both in average and in maximum length the
kokanee considerably exceed any group of residuals, although their range in
length overlaps that of the latter.   The largest kokanee approach the length of
the smallest third-year male anadromous sockeye, but in 1936 none of the
measured specimens of the latter were quite that small.

TABLE VIII.   Lengths in millimetres of kokanees taken.

Year	Number of specimens	Sex	Average length	Range	Standard deviation
1934	1	♂	223	–	–
1935	5	♂	339	315–365	19.6
"	5	♀	339	300–365	25.3
"	13†	♂ ♀	346	300–403	26.9
1936	1	♂	407	–	–
"	1	♀	396	–	–

†Includes the 10 previously listed.

The 1935 kokanees averaged about 500 grams (1.1 lb.) in weight (average
of 8 specimens 326 to 365 mm. long).   The male of 1936 weighed 737 grams
(1.6 lb.) after a year's preservation in formalin.   These weights appear to be
above the average of those in other British Columbia lakes (Dymond 1936).

COLOUR AT MATURITY

The colour of the kokanee taken closely resembled that of mature anadromous sockeye, or that of the lake Washington kokanee (Schultz loc. cit.). Both sexes were in fact rather more brightly coloured than the average Cultus lake anadromous sockeye. The males are very similar in having deformed jaws, enlarged teeth, humped backs and thin, deep bodies. The enlargement of jaws and teeth in the females tends to exceed that of sea fish of the same sex, and, combined with their unusually bright red colour, results in their being mistaken for males at first glance.

SUSCEPTIBILITY TO SALMINCOLA

In contrast to the progeny of anadromous sockeye, kokanee are little susceptible to attacks of these parasites, though whether by reason of constitution or habits is not known. In 1935 the infestation varied from none to four *Salmincola* on ten fish examined. In 1936 there were two small ones on the male, none on the female. In no case had the parasite developed egg-sacs. (Light infestations of *Salmincola* have been observed also on *Oncorhynchus kisutch* and *Salmo clarkii* from Cultus lake, but not on *Salvelinus malma*.) As far as is known, the only way in which an immature Cultus lake kokanee might be distinguished from an immature residual sockeye, would be by its unusually light infestation of these parasites. To date no such specimen has been obtained.

TIME OF SPAWNING

The time of capture of mature kokanee is shown in figure 5, being usually the last week of August and first of September. All of the specimens taken have been "dead" ripe, either exuding milt to the touch, or with eggs loose in the body cavity.

Time of spawning alone is sufficient to distinguish kokanee from other sockeye of the lake. It should be mentioned that at the time the kokanee were taken and for several months before, the outlet of the lake had been closed to the entrance of fish from downstream, hence there is no possibility that we are dealing with an early run of small marine sockeye.

SPAWNING HABITS

Mature kokanee, some of them with eggs partly spawned, have been captured at various places along the northeastern, eastern and southern shores of Cultus lake, so it is reasonable to believe that most of them spawn in the lake itself. The spawning pair observed in 1934 was in the lake, although near the outlet of a hatchery pond at Smith Falls. Reports of kokanee occasionally running into Smith Falls creek during past years are extant, but possible redds there have always been very limited, and of late years the water supply has become uncertain. A few may ascend the spring creek at the south end of the lake, but they have not been observed there.

Relation of Kokanee to Residual Sockeye

The discovery of residual sockeye in Cultus lake, and still more the discovery of differences between them and the kokanee, sheds new light upon the interesting question of the evolution of kokanee from anadromous sockeye. This matter is to be discussed in a separate paper now being prepared. For the present it is sufficient to observe that, although kokanee may have been derived from anadromous sockeye by way of the residual offshoots of the latter, the kokanee and residual populations in Cultus lake today are nearly or quite distinct. There has been no overlapping observed between them in regard to spawning colour, nor yet in infestation of *Salmincola*. In addition their spawning times are quite distinct: from observations to date it can be said that the last mature kokanee dies about three weeks before the first residual becomes ripe (figure 6). Although larger samples might possibly close this gap to some extent, it is clear that inter-breeding is negligible, if occurring at all.

Economic Importance

The scarcity of kokanee in Cultus lake precludes their being of any value or detriment to the lake at the present time. It is conceivable, however, that changes in the physical conditions of the lake resulting from present deforestation of the watershed, or changes in abundance of other fishes resulting from efforts to remove predators or from the introduction of new species, might act to greatly increase the kokanee population. In such an event, the probable importance of kokanee in the lake's economy would be as follows: as fingerlings and yearlings they would be used as food by game fish; in later years they would themselves be caught by anglers; at all ages they would compete with young anadromous sockeye for food; when large they might consume sockeye fry and fingerlings, or those of other valuable fish. In general, they would be occupants of an ecological niche at present filled (not necessarily to the saturation point) by the young of anadromous sockeye and their concomitant residuals. If this niche is capable of holding more occupants than at present, it is most desirable, from the economic standpoint, that the increase should be of sockeye rather than kokanee. Hence if kokanee ever show signs of increasing in the lake, such measures for their control should be undertaken as are economically feasible.

SUMMARY

1. Gill-netting of Cultus lake over a period of six years has revealed the presence of two types of non-anadromous sockeye salmon, here called residual sockeye and kokanee respectively. Distinguishing characteristics of the two are presented in the table on page 217.

Mature anadromous sockeye spawn at about the same time as the residuals, and their smolts are often similarly infested by *Salmincola*. Kokanee closely resemble anadromous sockeye in breeding coloration, whereas residuals do not.

2. A study of the sex and average rate of increase in length of the residual sockeye shows: (a) Residuals maturing in their second year grow more rapidly

in their first year of life than do most other residuals, or smolts; a small number of III and IV residuals, distinct from others of the same age by reason of very large size, have a first growth similar to that of II residuals.   (b) Most residuals maturing in their third or fourth year of life have grown more slowly in their first year than have year-old smolts.   (c) Most residuals maturing in their fourth year have grown more slowly in their second year of life than have those maturing in their third year.   (d) Second-year residuals, and the very large III's and IV's, are entirely male; third-year residuals include a small proportion of females; and fourth-year a probably larger proportion.   The segregation of the progeny of anadromous sockeye into residual and anadromous groups is therefore influenced by their size and sex.   The various types found are indicated in figure 5.

Characteristic	Residual sockeye	Kokanee
Sex ratio	Predominantly male	Normal
Age at maturity	Males: II, III, less often IV	Males: III and IV (?)
	Females: III and IV	Females: IV (?)
Size at maturity	16 to 38 cm.	22 to 41 cm.
Colour at maturity (males)	Dull olive-grey	Green head, red body
Absorption of scale margins at maturity	Little or none	Always advanced
Infestation of Salmincola	Usually heavy	Light or none
Time of spawning	October to December	August and September
Parentage	Anadromous sockeye	Presumably other kokanee
Abundance	Usually common	Scarce

3. The food of residuals in their second or third year of life resembles that of fingerling sockeye, being largely plankton Crustacea, with some midges and terrestrial insects.   Fourth-year residuals consume very small fish to some extent, including newly-emerged sockeye.

4. As competitors of and occasionally predators upon young anadromous sockeye, the presence of residual sockeye in the lake may be considered detrimental to its most efficient utilization as a nursery for fish of commercial value. By analogy, kokanee may be considered equally detrimental in proportion to their numbers, but their scarcity makes them of little importance at the present time.

5. Although kokanee in Cultus lake may well have originated from the residual type of sockeye, they appear now to be a population distinct from the latter and from the anadromous fish.

ACKNOWLEDGEMENT

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The Influence of Hydrogen-ion Concentration on Some Acid Resistant Bacteria

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ABSTRACT

Fifty-three strains of bacteria have been isolated from acidulated brine. They are classified into *Bacillus*, *Micrococcus*, *Sarcina*, and *Lactobacillus*. Of these the *Bacillus* strains failed to grow at hydrogen-ion concentrations more acid than pH 6.20, while the *Micrococcus* and *Sarcina* forms showed a greater tolerance, producing limited growth from pH 5.22 to 5.64. Other workers describing wider limits for the genus *Bacillus* failed to allow for the change produced in the weakly buffered environment by the living cell. The microaerophilic *Lactobacilli* grew at pH 3.52. These aciduric types are not actively proteolytic.

While studying the deterioration of a marine product consisting of herring (*Clupea harengus*), in acidulated brine, 53 bacterial strains were isolated. These bacteria were viable after resisting hydrogen-ion concentrations varying from pH 3.8 to 4.6 for several months. In order to find the significance of these bacteria from the point of view of decomposition, a study was made of their metabolism and their power to tolerate or to resist acid environments under laboratory conditions.

In any study of this character certain variables in the medium, other than hydrogen-ion, must be considered in determining the degree of acid tolerance. Sherman and Holmes (1922) have demonstrated that stimulating concentrations of sodium ions extend the acid tolerance of some bacteria. It has also been shown that certain cations and anions may induce an antagonistic effect at levels of concentration above the stimulating range (Reed 1924; Falk 1923; Winslow 1934). It is also important that the buffering power of the medium be sufficient to maintain the pH value at an approximately constant level so that incipient metabolic activity may not shift the hydrogen-ion concentration to a more favourable range. This factor has been too frequently neglected in previous studies (Dernby 1922). These factors were taken into consideration in devising a highly buffered nitrogenous medium of constant composition except for the hydrogen-ions as given below.

In this paper the limits of acid tolerance of the organisms have been determined in the sense of Rosebury (1932) and distinguished from resistance to acid environments by means of a suspended controlling mechanism.

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METHODS

ISOLATION AND IDENTIFICATION

Aerobes were isolated on beef extract agar containing 1.5 per cent sodium chloride. The anaerobic technique developed by Spray (1930 and 1936) was successfully applied to the culturing of the microaerophilic *Lactobacilli*. The morphological and cultural characteristics were determined for each strain. The classification used was that of Lehmann, Neumann and Breed (1931) and Bergey (1934).

Forty strains belonging to the family *Bacillaceae* were isolated. Of this family 26 strains were members of the *Mesentericus* group, 7 strains were *B. cereus* and 7 strains could not be classified. Belonging to the family *Coccaceae*, 3 distinct species of *Micrococcus* and 2 species of *Sarcina* were isolated. The microaerophilic strains were all of the same general type. A cultural study showed them to be gram positive, non-spore-forming rods. They were tentatively classified and grouped according to Schlirf (Lehmann, Neumann and Breed 1931) under the *Lactobacilli* of Bergey (1934). These organisms resembled those recently isolated from fish intestines by Shewan (1937).

PREPARATION OF MEDIA

Two types of media were employed. For the aerobes, the peptone medium of Hansen (1930) in agar slants was utilized. The microaerophiles were cultured in the semi-solid medium of Spray (1936). All hydrogen-ion concentration determinations were made with the aid of a vacuum tube potentiometer in conjunction with a Morton glass electrode. Throughout the investigation the absolute experimental error was 0.05 pH with a relative accuracy of 0.02.

The media were buffered with acetic acid and sodium acetate in the same proportions as used by Walpole (1914). Normal solutions of the acid and salt were added to the media so that the final concentration of acetate at any pH was 0.2N. The data in figure 1 were determined experimentally. To buffer the media at various hydrogen-ion concentrations between pH 3.5 and 6.6 the required amounts of acid and salt were determined from the graphs. The curves were sufficiently accurate to enable an adjustment to within the desired range of hydrogen-ion concentration. After the addition of the buffer the exact acidity was determined by means of the glass electrode. It is evident from figure 1 that the buffering capacity of the acetate-acetic acid system decreases rapidly as the hydrogen-ion concentration approaches pH 3. It is also apparent that the constituents of Spray's medium become very efficient buffers within this range. Therefore, to adjust the medium below pH 4.5 it was necessary to add normal hydrochloric acid in addition to the sodium acetate-acetic buffer. The nutrient constituents of the media were sterilized by heat, the buffer solutions were passed through Seitz E. K. filters, after which they were added to the medium from sterile burettes.

The only variable in the media other than the hydrogen-ion was the sodium ion, the maximum concentration of which was 0.4 M. Preliminary tests showed that the organisms under investigation were slightly inhibited only at sodium ion

concentrations of 0.7 M. Therefore it was concluded that in these culture media the only significant variable was the hydrogen-ion concentration.

GROWTH

In determining the acid tolerance, the aerobic cells were inoculated on the test media from 24 hour slants made up from Hansen's peptone medium and adjusted to pH 6.5. Each test slant was inoculated uniformly with a 4 mm. loopful of cell suspension. Incipient growth could be detected, the organism being confined to a thin layer over the surface. The microaerophiles were inoculated into series of culture tubes containing Spray's semi-solid medium. Growth was readily detected by turbidity.
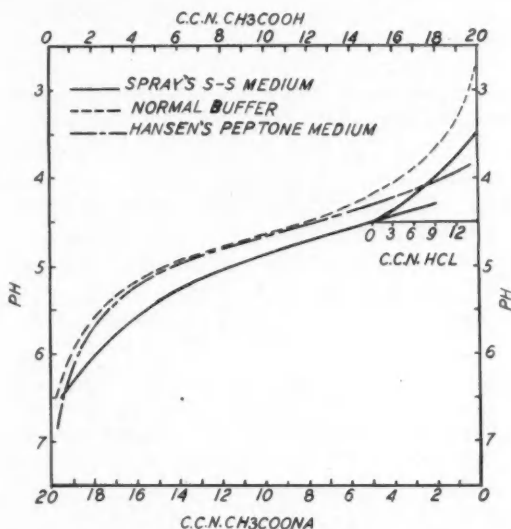
FIGURE 1. Curves used in the adjustment of the media.

PROTEOLYSIS

As a qualitative index of metabolic activity the production of ammonia was selected to indicate the utilization of peptones. The test developed by Hansen (1930 and 1935) has been used throughout the investigation. This reaction has been reported by Hansen, and also by Lapin and Hein (1934) to be sensitive to ammonia to one part in a million. In order to render the test more sensitive the medium was extracted with ether, the density of the colour in the solvent being directly proportional to the concentration of ammonia.

RESULTS WITH AEROBIC BACTERIA

For each strain isolated, observations on growth and ammonia production were made over a range of pH 6.50 to 4.00, with an incubation period of 20 days at 30°C.

Table I contains a summary of these observations. All species tested grew profusely at pH 6.80. The strains belonging to the genus *Bacillus* showed a very limited tolerance to hydrogen-ions. The highest concentration tolerated was 6.20. A typical growth reaction has been observed. At pH 6.20 there was a decided inhibitive effect during the early part of the incubation period among the members of the *Mesentericus* group. In nearly all cases growth started at the very thin part of the slant, progressing slowly toward the base for the first 10 days of incubation. At this point growth was rapid until there was an abundant growth over the whole length of the slant. In the case of these *Bacillus* strains there was a marked neutralization effect by the production of basic substances, as shown by the final hydrogen-ion concentrations recorded in table I. The very strong ammonia tests were conclusive proof that the neutralizing mechanism was the result of the production of this basic substance.

TABLE I. Hydrogen-ion concentration tolerance and ammonia production of aerobic bacteria between the limits pH 6.80 and 4.24. Incubation for 20 days at 30°C.

Organism	Minimum pH showing growth	Hansen's ammonia	Final pH (Initial pH 6.80)
B. aterimus	6.20	+++	9.34
B. mesentericus			
6 strains	6.80	+++	9.12
5 strains	6.20	+++	
B. vulgatus			
11 strains	6.20	+++	9.15
3 strains	6.80	+++	
B. cereus			
2 strains	6.20	+++	8.73
5 strains	6.80	+++	
M. pyogenes albus	5.22	++(pH 6.00)	8.98
M. candicans	6.00	+	8.36
M. sulfureus	5.64	+++(pH 6.20)	9 40
S. flava	5.64	++(pH 6.20)	9.34
S. alba	5.64	+(pH 5.90)	5.92

+Weak ammonia production.
++Medium ammonia production.
+++Strong ammonia production.

Members of the family *Coccaceae* were slightly tolerant to hydrogen-ions. At the maximum acidities tolerated, however, the amount of growth was limited. With the exception of *S. alba*, the group was very proteolytic at reactions more

basic than pH 6.00. *S. alba* was acidogenic and slightly proteolytic at all hydrogen-ion concentrations tolerated.

RESULTS WITH LACTOBACILLI

The aciduric properties of this group are well established in the literature, but the extent of their proteolytic activity when cultured in nitrogenous media has not been entirely confirmed.

Since the organisms under investigation were isolated from an acidulated protein environment, the present experiment was directed toward a study of the following : the acid tolerance limits for the group ; and the amount of proteolysis at the various hydrogen-ion concentrations tolerated.

ACID TOLERANCE

The acid tolerance limits of this group were determined by inoculating 0.1 cc. of a 48 hour culture into series of Spray's semi-solid medium, buffered at hydrogen-ion concentrations between pH 3.52 and 6.64. The results tabulated in table II show that the group was extremely acid tolerant. Growth was profuse at all hydrogen-ion concentrations tolerated. *L. bulgaricum* was tolerant at pH 3.52; the critical tolerance was not determined. Within the group there was a single exception,—*L. aerogenes* did not tolerate a hydrogen-ion concentration more acid than below pH 6.20. These results are in fair agreement with other authors reporting on this genus, Rosebury (1932), and Weiss and Rettger (1934).

TABLE II. Acid tolerance tests on *Lactobacilli* in Spray's anaerobic medium and ammonia production on peptone slants cultured in McIntosh-Fildes anaerobic jar. For minimum pH, the range in pH was from 6.64 to 3.52, and incubation was for 20 days at 30°C. For Hansen's ammonia, the range in pH was from 6.50 to 4.50, and incubation was for 10 days at 30°C.

Strain	Minimum pH showing growth	Hansen's ammonia
L. acidophilum (2)......	3.94	−
L. acidophilum (3)......	4.28	−
L. aerogenes...........	6.20	−
L. species (5).........	4.10	−
L. species (4).........	3.94	++
L. bulgaricum group 3 strains...........	3.52<	++

++Medium ammonia production.
−No ammonia production.

PROTEOLYSIS

Weinstein and Rettger (1932) have demonstrated *Lactobacilli* utilizing peptones. The test was based on nitrogen utilization necessary for growth, therefore the result was not an index of the extent of proteolysis. Since it is well established in the literature that this genus is acidogenic and rapidly reaches the limiting pH tolerance in unbuffered or uncontrolled media (Weinstein and Rettger 1932; Rosebury 1932; Weiss and Rettger 1934; Longsworth and McInnis 1936), it is apparent that any proteolytic characteristics of the organisms would be masked by the active acid production. In other words the acidity of the medium reaches the limiting value for growth before the carbohydrates are completely utilized. Therefore, in determining the proteolytic characteristics for these organisms, well buffered nitrogenous media containing small quantities of carbohydrates were employed.

Tests for proteolysis similar to those carried out for aerobic species have been recorded in table II. These tests were carried out under anaerobic conditions since the organisms failed to grow in the presence of air. Two members of this group gave positive tests for ammonia, indicative of proteolysis. Ammonia was produced by these species at all hydrogen-ion concentrations tested between pH 4.5 and 6.6.

To test the extent of proteolysis an experiment was carried out based on the fact that in a nitrogenous medium, an active nitrogenous metabolism including proteolysis would produce a significant decrease in hydrogen-ion concentration. This reaction has been demonstrated among the aerobic species belonging to the genus *Bacillus*. The experiment recorded in table III shows that Spray's semisolid medium inoculated with members of the *Lactobacilli* increased in hydrogen-ion concentration for the first five days of incubation. The medium was sufficiently buffered to prevent the reactions from reaching the limiting values for growth. A continued record of hydrogen-ion concentration showed that in some strains no further change occurred after the first five days; in other strains there was a slow decrease in the hydrogen-ion concentration, up to 20 days. This decrease after the first five days has been recorded as an index of nitrogen metabolism. The values recorded as x in table III are the changes in pH up to five days; the y values are the changes occurring after 20 days. It is evident that the difference between x and y is directly proportional to the changes produced by nitrogenous metabolic products. The values recorded under $(x-y)$ in table III indicate that *L. bulgaricum*, *Lactobacillus sp.* 4 and *L. aerogenes* are capable of breaking down nitrogenous constituents with the production of basic end products. These results confirm the findings in table II, with the exception of *L. aerogenes*, which produced no ammonia on Hansen's peptone medium. The discrepancy may be accounted for by the incipient growth on the peptone medium as compared to the profuse growth on the Spray medium, which undoubtedly contains more available nitrogenous constituents. This experiment was repeated at higher hydrogen-ion concentrations between pH 6.64 and 3.52. Confirmatory results were obtained at all hydrogen-ion concentrations. The values for x and y became smaller as the hydrogen-ion concentration was increased toward the

TABLE III.   Reaction changes in Spray's semi-solid medium as an index to the *nitrogen metabolism* of *Lactobacilli*.

Strain	5 days at 30°C.			20 days at 30°C.			
	pH control	pH final	pH change-x	pH control	pH final	pH change-y	x-y
L. acidophilum (2)	6.64	6.00	-0.64	6.60	5.98	-0.62	0.00
L. acidophilum (3)	6.64	6.02	-0.62	6.60	5.96	-0.64	0.00
L. aerogenes	6.64	6.25	-0.39	6.60	6.46	-0.14	+0.25
L. species (5)	6.64	6.14	-0.50	6.60	6.10	-0.50	0.00
L. species (4)	6.64	6.26	-0.38	6.60	6.34	-0.26	+0.12
L. bulgaricum group							
Strain 1	6.64	6.14	-0.50	6.60	6.28	-0.32	+0.18
" 2	6.64	6.16	-0.48	6.60	6.30	-0.30	+0.18
" 3	6.64	6.16	-0.48	6.60	6.22	-0.38	+0.10

Among the x-y values differences of less than 0.04 pH between x and y have been recorded as 0.00 since this change is within the experimental error of the method.

limiting pH values for growth.   On comparing the x-y values which represent nitrogen metabolism with the x values which indicate the utilization of a small amount of carbohydrate, it is evident that the *Lactobacilli* under investigation were not actively proteolytic.

DISCUSSION

Several investigators have found that bacteria are capable of changing their environment suitable to the life of the cell.   Bruce (1935) working with *B. pyocyaneus* cultured in weakly buffered media, found that the organisms grew poorly at pH 5.0.   Slow growth resulted in a decrease in hydrogen-ion concentration, which made the reaction favourable for growth.   Shaughnessy and Winslow (1927) have shown that certain cells exert a distinct influence upon the reaction of the menstrum.   It is apparent, however, that such a reaction has not been considered when determining the hydrogen-ion concentration tolerances for many bacterial species.   Thus Dernby (1922), has recorded the pH limits of growth for *B. subtilis*, a species closely related to *B. cereus*, between pH 4.5 and 8.5.   Stearn and Stearn (1933) have recorded *B. subtilis* growing at a minimum pH of 5.1.   They have pointed out that the organism forms alkali readily, but they have failed to record the final hydrogen-ion concentration.   These workers carried out their investigations on lightly buffered media which lend themselves to rapid changes.   Therefore, the importance of *maintaining* the medium at definite hydrogen-ion concentrations by the addition of acids or buffering must not be overlooked in determining hydrogen-ion concentration tolerances for bacteria.   The discrepancy between the narrow tolerance limits as recorded for the genus *Bacillus* in this paper and the

relatively wide limits as recorded in the literature may be accounted for in the light of this consideration.

The members of the family *Coccaceae* that were examined were slightly acid tolerant, resisting hydrogen-ion concentrations in the range pH 5.22 to 5.94. At these concentrations, however, growth and proteolytic activity were limited. They cannot be classified as acid tolerant bacteria.

The aciduric, microaerophilic strains tentatively classified under the *Lactobacilli* were very acid tolerant. A few species of this group maintained active growth at pH 3.52. From a record of reaction changes in buffered nitrogenous media at all hydrogen-ion concentrations tolerated, and also from the results of ammonia tests on peptone media it has been concluded that members of this group are not sufficiently proteolytic to produce significant decomposition.

ACKNOWLEDGEMENT

SUMMARY

Fifty-three strains of aerobic and microaerophilic bacteria resisting acid environments have been isolated and classified under the following genera: *Bacillus*, *Micrococcus*, *Sarcina* and *Lactobacillus*.

Hydrogen-ion concentration tolerances have been determined in carefully controlled experiments throughout the limits pH 3.5 to 6.5.

All aerobic species were found to be non-acid tolerant while the microaerophilic species cultured under anaerobic conditions exhibited extreme tolerances to acidity.

Members of the genus *Bacillus* grow over a narrower range of hydrogen-ion concentration than formerly recorded in the literature for species of this genus. A reaction which may account for the discrepancy has been discussed.

The aciduric strains were not sufficiently proteolytic to be of significance in decomposition processes at high hydrogen-ion concentrations.

The microaerophiles were aciduric types. Their metabolism was actively saccharolytic while 3 strains were weakly proteolytic.

Among the aerobic and microaerophilic species ammonia production as an index of proteolysis was found to be a function of growth.

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